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THE USE OF SOCIAL MEDIA TO DOCUMENT A RANGE EXTENSION OF AN ICONIC SOCIAL PARASITE ANT

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Ants of the genus *Polyergus* Latreille are large (4.8–7.5 mm), brightly coloured daytime active ants from numerous locations across the Holarctic with obligate social parasitic behaviour that includes highly visible mid-afternoon "raids" conducted against species of *Formica* Linnaeus that has entrenched their iconic status amongst many myrmecologists (Trager 2013). Accordingly, their presence in an area is often well documented. Thus, a novel location, especially a northern range extension, is worth reporting. We describe here one such interesting range extension that was identified and confirmed with the assistance of social media.

Ants of the species *Polyergus lucidus* Mayr were collected from Killbear Provincial Park (45.3589, -80.2134), near Parry Sound Ontario by McDonald and Kent in late August 2016. The previously known most northerly collection in Canada was made by Gary Umphrey at Pinery Provincial Park, near Grand Bend Ontario (43.2519, -81.8276). While Ellison *et al.* (2012) observed that *P. lucidus* specializes on *Formica incerta* Buren, the host ant species was neither observed nor collected at Killbear. The Killbear specimens of *Polyergus* were collected in the disturbed areas adjacent to the visitors centre parking lot and then shipped to the University of Guelph where Smith pointed and photographed the ants with the assistance of Smith lab students and volunteers using a Leica Z16 APOA Macroscope and the Leica Application Suite V4.3. These photos allowed the species identification to be confirmed (Fig. 1).

In a recent revision, Trager (2013) resurrected many species that Creighton (1950) had previously synonymised into species with broad geographic distributions. Based on this revision, our contemporary understanding of the species of *Polyergus* is now more geographically associated. The collection reported here represents a range extension of approximately 270 km to the north-east for *Polyergus lucidus* (Fig 2, Table 1). In an attempt

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to contextualise this discovery, we mined abiotic variables from the WorldClim dataset (Hijmans *et al*. 2005) by extracting the approximate latitude and longitudes of the specimens examined in Trager (2013). The Killbear site is not the most northerly site (a site in northern Wisconsin is slightly farther north), however in many ways it is unlike much of the other locations examined by Trager (2013) (Table 1). For example, the mean annual temperature of Killbear is approximately one degree Celsius colder than Pinery (the most northerly previously known Canadian site). At this point, we do not possess sufficient data for a clear prediction of the thermal profile of *P. lucidus*; however, further reported occurrences such as those we describe here might suggest that the range of *P. lucidus* in Canada is expanding with a warming climate.

The confirmation of this collection at a novel locality was made possible via the social media platform, Twitter (Fig 3). On August 31st, 2016 McDonald and Kent tweeted a series of posts asking for identification help with biting ants collected in Killbear Provincial Park. Based on the colour and aggressive nature of the ants, the authors posted the tweets to see if these were European fire ants, *Myrmica rubra* (Linnaeus). The initial tweet was amplified by Morris who suggested that Smith might help with identification. Kent began taking cell phone photos through his reversed binoculars which made it immediately clear that these were *Polyergus* and not *Myrmica* fire ants. However, species-level identification from these photos was not possible. Kent shipped the ants to Smith at the University of Guelph so that a more definitive identification could be made. Trager examined the initial photos in the tweets and the eventual z-stacked photos and confirmed the identification of *P. lucidus.*

We describe here a case in which social media (Twitter) has enabled the identification of a range extension of a large and charismatic temperate ant. The use of social media platforms such as Twitter may become more prevalent and important in the future to rapidly document and share range extensions or species translocations (Daume and Galaz 2016; Jackson *et al*. 2016). Based on changing latitudinal isotherms, we consider it likely that the northern extent of *Polyergus lucidus* has not yet been discovered or reached. Twitter and other forms of social media and citizen science will likely be involved in documenting and sharing these new discoveries.

FIGURE 1. Frontal and lateral view of one of the *Polyergus lucidus* specimens collected in Killbear Provincial Park, August 2016.

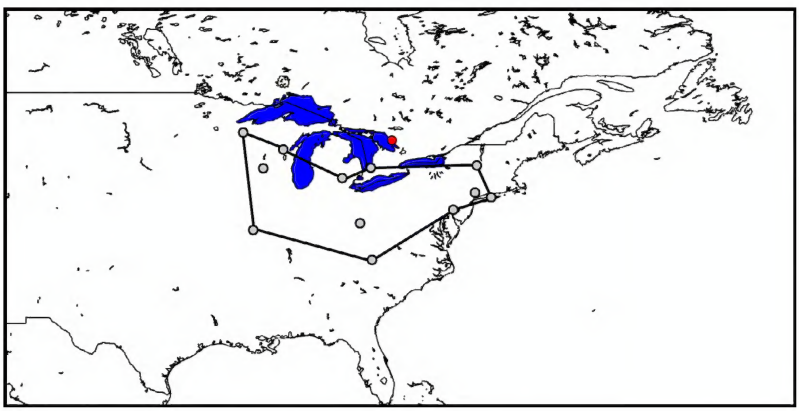

FIGURE 2. Range extension of *Polyergus lucidus* illustrated on a map of eastern North America. The collection detailed here (in red) is compared to the specimens examined by Trager (2013) (in grey) contained in a minimum convex polygon connecting Trager-examined specimens. The map was made by plotting latitude and longitude values obtained from Google for the examined samples in SimpleMappr (http://www.simplemappr.net/).

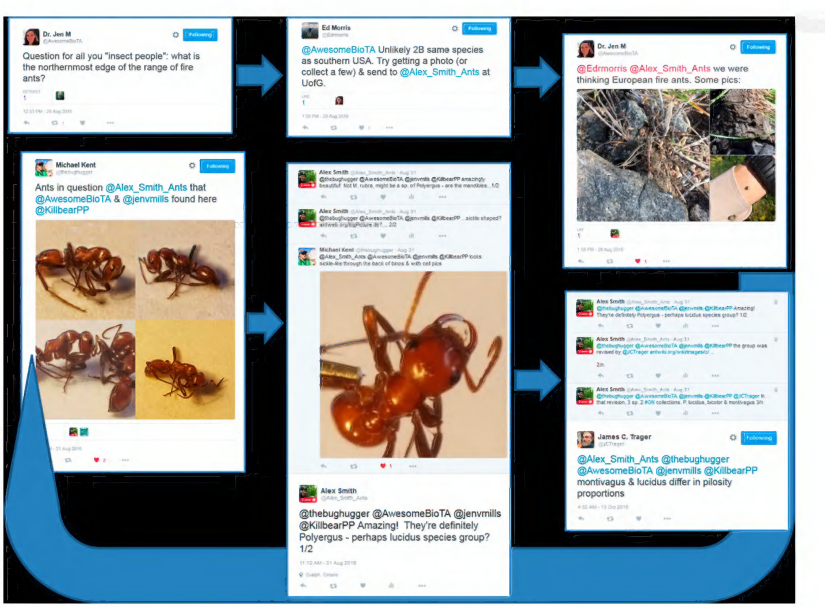
Question for all you "insect people": what is the northernmost edge of the range of fire ants?
@AwesomeBioTA Unlikely 2B same species as southern USA. Try getting a photo (or collect a few) & send to @Alex_Smith_Ants at UofG.
@Edrmorris @Alex_Smith_Ants we were thinking European fire ants. Some pics:
Ants in question @Alex_Smith_Ants that @AwesomeBioTA & @jenvmills found here @KillbearPP
@thebughugger @AwesomeBioTA @jenvmills @KillbearPP Amazing! They're definitely Polyergus - perhaps lucidus species group? 1/2
@Alex_Smith_Ants @thebughugger @AwesomeBioTA @jenvmills @KillbearPP montivagus & lucidus differ in pilosity proportions

FIGURE 3. Archive of tweets regarding the collection of the ants and helping to confirm their eventual identification (https://dx.doi.org/10.6084/m9.figshare.4542625). Specific tweets have been compiled using Storify: https://storify.com/Alex_Smith_Ants/polyergus-ants-from-killbear-provincial-park

TABLE 1: Collection localities and the annual mean temperature for each location for *Polyergus lucidus* samples examined by Trager (2013) and here. Latitude and longitude were derived from Google searches of the locality information from Trager (2013), and then DIVA-GIS (Hijmans *et al.* 2001) was used to mine the WorldClim (Hijmans *et al.* 2005) dataset for five abiotic variables associated with the sites.

Sites	Lat.	Long.	BIO1 = Annual Mean Temp.	BIO2 = Mean Diurnal Range (Mean of monthly (max - min temp.))	BIO5 = Max Temp. of Warmest Month	BIO11 = Mean Temp. of Coldest Quarter	BIO12 = Annual Precip.
ONTARIO Killbear Provincial Park	45.3589	-80.2134	5.433	10.867	25.2	-1.350	1015
ONTARIO Pinery Provincial Park	43.2519	-81.8276	6.283	12.567	26.2	-0.350	891
WISCONSIN Navarino State Wildlife Area	44.6442	-88.5275	6.154	12.308	27.7	-1.467	787
WISCONSIN Spring Green Preserve State Natural Area	43.2062	90.0583	6.858	13.717	28.5	-0.817	810
MICHIGAN U of M Edwin S. George Reserve	42.4585	-84.0115	6.879	13.758	27.7	-0.117	791
MISSOURI Shaw Nature Reserve	38.4821	-90.8245	9.588	19.175	31.9	2.967	990
NEW YORK Putnam Co., Putnam valley	41.3424	-73.8491	7.854	15.708	28.9	1.467	1183
NEW YORK Suffolk Co.	40.9849	-72.6151	7.679	15.358	27.2	2.100	1143
NEW YORK Warren Co., Lake George	43.4262	-73.7123	6.392	12.783	26.7	-0.367	1078
NORTH CAROLINA Watauga Co.	36.1942	-81.7349	8.071	16.142	25.7	3.183	1438
OHIO Jackson Co.	39.006	-82.6483	8.879	17.758	29.4	2.633	1043
PENNSYLVANIA Chester Co., Malvem	40.0362	-75.5138	8.258	16.517	29	2.067	1153
WISCONSIN Washburn, Bass Lake	45.95	-91.5928	5.738	11.475	27.5	-2.317	772

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COMPARATIVE TOXICITY OF THE NONSTEROIDAL ECDYSONE AGONISTS TEBUFENOZIDE AND METHOXYFENOZIDE TO EARLY AND LATE LARVAL INSTARS OF THE WHITE-MARKED TUSSOCK MOTH, *ORGYIA LEUCOSTIGMA*

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Abstract

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Insecticidal toxicity can vary widely as a function of the developmental stage of the insect being targeted. Here the toxicity of the nonsteroidal ecdysone agonists tebufenozide and methoxyfenozide was evaluated against early and late instars of the white-marked tussock moth, *Orgyia leucostigma* (J.E. Smith) (Lepidoptera: Erebidae), using a droplet-feeding bioassay. On the basis of LD_{50} estimates, methoxyfenozide was ~9 and 22 times more effective than tebufenozide in inducing mortality in 1st and 4th instars of *O. leucostigma*, respectively. Unlike methoxyfenozide, tebufenozide was unable to cause more than 70% mortality in 4th instars, even at the highest dose tested (1 µg/larva). Analysis of the present data suggests that susceptibility of late instar larvae to tebufenozide is significantly compromised whereas that to methoxyfenozide is much less so, which suggests that the mechanism conferring resistance to tebufenozide in late instar larvae is either ineffective or less effective in the case of methoxyfenozide.

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Introduction

Nonsteroidal ecdysone agonists of the diacylhydrazine family comprise several insecticidal molecules that can induce a precocious and lethal larval molt. Their mode of action involves high-affinity binding to the ecdysone receptor and the persistent expression of molt-related transcription factors (Dhadialla *et al.* 1998; Nakagawa 2005; Retnakaran *et al.* 2001). Two such compounds, tebufenozide (RH-5992; Smagghe and Degheele 1994) and methoxyfenozide (RH-2485; Carlson *et al.* 2001), are highly selective for lepidopterans

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and, as such, are currently registered in Canada for the control of various lepidopteran pests; however, only tebufenozide has been registered for the suppression of forest insect pests. Because of their specificity to lepidopterans, these two growth regulators have minimal impact on non-lepidopteran organisms and are therefore considered reduced-risk insecticides.

Differences in susceptibility among insect life stages to a given insecticidal compound have often been reported, where early instars are typically more susceptible than later ones (Robertson *et al.* 2007). Such instar-associated variation in susceptibility to diacylhydrazines has been observed for several species, including examples where early instars were more susceptible than later instars (Moulton *et al.* 2000; Knight *et al.* 2001) and vice versa (Smagghe and Degheele 1994; Sáenz-de-Cabezón Irigaray *et al.* 2005), as assessed by comparing LC_{50} values estimated using leaf-dip or diet incorporation assays. The white-marked tussock moth (WTM), *Orgyia leucostigma* (J.E. Smith) (Lepidoptera: Lymantriidae), a highly polyphagous pest responsible for significant defoliation in deciduous and coniferous forest stands in Canada (Dedes 2009), may well represent an extreme example of developmental variation in susceptibility to tebufenozide. Indeed, first instars were reported to be highly susceptible to this compound, while 4th and 5th instars seemed largely resistant to it (Retnakaran *et al.* 2001, 2003). Following treatment with µg doses, 4th instars entered a moribund state from which they recovered after several days and moulted to the 5th stadium. It has been hypothesized (Retnakaran *et al.* 2003) that this late-instar resistance results from the cells' ability to pump out the analog using an ABC transporter homologous to the one identified in yeast (Hu *et al.* 2001).

In an effort to further explore the biochemical basis of this type of resistance and to identify genes whose transcription may be modulated differently by diacylhydrazines in different developmental stages, we undertook a study focusing on the transcriptional analysis of *O. leucostigma* larvae treated as 1st or 4th instars with either tebufenozide or methoxyfenozide. Given that the latter compound is typically effective at lower doses than the former (e.g., Carlson *et al.* 2001), we wanted to determine whether it was subject to the same type of developmental resistance reported for tebufenozide. Because LD_{50} toxicity of these two growth regulators to *O. leucostigma* has never been formally assessed, we estimated the LD_{50} values for the two compounds and developmental stages, so that we could later standardize treatments and dose acquisition for our transcriptional analyses. Here, we report on the results of these toxicity assessments.

Materials and Methods

Chemicals

Technical grade tebufenozide (N-tert-butyl-N-3,5-dimethylbenzoyl-N'-4-ethylbenzoylhydrazine; 95%) and methoxyfenozide (N-tert-butyl-N-3,5-dimethylbenzoyl-N'-3-methoxy-2-methylbenzoylhydrazine; 95%) were obtained from Dow AgroSciences (Indianapolis, USA). Analytical grade acetone was used in the preparation of the insecticidal solutions.

Insects

Larvae of *O. leucostigma* and the Bell artificial diet (Bell *et al.* 1981) on which they were reared were obtained from Insect Production Services, Canadian Forest Service, Sault Ste. Marie, Ontario (http://www.nrcan.gc.ca/forests/research-centres/glfc/13467). Larvae were reared individually in 22 mL plastic cups at 22°C, 50% relative humidity, and under a 12h:12h light:dark photoperiod. Male and female larvae were left unseparated. Larvae were transferred onto fresh Bell diet every 2 weeks.

Larval toxicity assays

LD_{50} values were estimated for both tebufenozide and methoxyfenozide in 1-day-old 1st and 4th instar larvae. Prior to treatment, larvae were starved individually for 24 h in empty diet cups. For dose acquisition, we used a droplet-feeding method (van Frankenhuyzen *et al.* 1997; Dallaire *et al.* 2004). Serial dilutions of the compounds were prepared in distilled water containing 1% acetone, 2% sucrose, and 2% red food colouring (Club House). Control larvae were fed the carrier only (i.e., the solution used for dilutions).

Five doses varying between 1.125×10^{-2} and 112.5 ng of tebufenozide or methoxyfenozide were administered to 1st-instar larvae in a volume of 0.25 µL of carrier deposited at the bottom of a Fisherbrand 0.25 mL microfuge tube, using a Cole-Parmer 74900 series microinjector mounted with a 0.25 cm^3 tuberculin syringe equipped with a 31-gauge Hamilton needle. A micro magnetic stirring bar (7×2 mm) was used to keep the insecticide in solution within the syringe. Five (methoxyfenozide) and six (tebufenozide) doses varying between 0.1 and 1125 ng of the same compounds were administered to 4th-instar larvae in a volume of 2.5 µL deposited at the bottom of a 12×75 mm culture tube (5 mL) using a P-10 Gilson pipette.

One larva of the appropriate stadium was transferred to each droplet-containing tube. Tubes were then held upside down under a fluorescent light for 4 h, and only larvae that imbibed the whole droplet during this period were used in the experiment. Thirty 1st and twenty 4th instars were processed in this way for each insecticide and each dosage, including control conditions. After imbibing the droplet, larvae were transferred to fresh Bell diet and held under the conditions described above. To assess mortality, larvae were monitored daily for up to 2 weeks following treatment. Insects that did not move after being probed with the tip of a fine paintbrush were considered dead.

Larval mass

To examine the relationship between larval mass and the estimated LD_{50} values, fifteen 1st- and ten 4th-instar larvae (1 day old) were weighed using a Mettler AE 100 balance.

Probit analysis

For probit analysis (Bliss 1934), we used the StatPlus Pro 2009 software from AnalystSoft Inc. (www.analystsoft.com). LD_{50} values (the amount of material, given all at once, that causes the death of 50% of a group of test animals) were estimated for each compound and each developmental stage.

Results

In comparing LD_{50} estimates obtained for the two test compounds, methoxyfenozide was ~9 and 22 times more effective than tebufenozide in causing mortality in 1st and 4th instars of *O. leucostigma*, respectively (Table 1). Strikingly, the 4th instar:1st instar LD_{50} ratio was more than twice as high for tebufenozide (~28) than for methoxyfenozide (~11), pointing to a difference between the two growth regulators with respect to the development of resistance in older instars.

Considering the existence of differences in size among larvae of different developmental stages, an increase in the dose required to achieve mortality in 4th instars relative to 1st instars was not completely unexpected. To examine the relationship between larval mass and LD_{50} estimates, the latter values were divided by the average mass of 1st- and 4th-instar larvae (0.316 ± 0.003 mg and 47 ± 8 mg, respectively). Interestingly, on a per mg basis, 4th instars appeared more susceptible than 1st instars to either compound, although this difference was greater for methoxyfenozide (14×) than for tebufenozide (5×), as determined from LD_{50} estimates (Table 1).

Discussion

In the present study, we conducted an assessment of the toxicity of two diacylhydrazine insecticides, tebufenozide and methoxyfenozide, against 1st and 4th instars of *O. leucostigma*. Originally meant as a preliminary step aimed at selecting appropriate doses (LD_{50}'s) for the treatment of larvae to be used in a transcriptional study, the work presented here already sheds some light on the phenomenon of developmental resistance to tebufenozide reported earlier for late instars of *O. leucostigma* (Retnakaran *et al.* 2001, 2003).

TABLE 1: Probit statistics, including LD_{50} estimates (ng/larva), for the toxicity of tebufenozide and methoxyfenozide estimated for 1st and 4th instars of the white-marked tussock moth, *Orgia leucostigma*, using a droplet-feeding assay. The last column shows LD_{50} estimates expressed as ng/mg.

Insecticide	Instar	Slope ± SD	Intercept	LD_{50}	(95% CL)	LD_{50} (ng/mg)
tebufenozide	1st	1.3 ± 0.8	3.3	19.0	(0.7; 523)	60.2
	4th	0.7 ± 0.2	3.2	538.5	(163; 4713)	11.5
methoxyfenozide	1st	1.5 ± 0.4	4.4	2.2	(0.2; 45)	7.0
	4th	1.5 ± 0.3	3.0	24.2	(12.5; 47)	0.5

Although the 95% confidence limits determined for the LD_{50} estimates presented here point to a significant degree of variability in the responses of *O. leucostigma* to the test compounds (Table 1; more pronounced for tebufenozide than for methoxyfenoside, in part because of the poor response of 4th instars), our data indicate that the relationship between developmental stage and susceptibility to the test compounds differs for tebufenozide and methoxyfenozide. Not only was methoxyfenozide overall more effective than tebufenozide, as shown previously for other lepidopteran species (Charmillot *et al.* 2001; Trisyono and Chippendale 1998; Knight *et al.* 2001; Borchert *et al.* 2004; Moulton *et al.* 2000; Smagghe *et al.* 2000; Sundaram *et al.* 1998), it was considerably more potent against 4th instars than tebufenozide. For example, the L4:L1 LD_{50} ratio was 2.5 times higher for tebufenozide than for methoxyfenozide (see Results and Table 1). In addition, the highest tebufenozide dose tested against 4th instars (1 µg/larva) was insufficient to kill all or most larvae, which confirms earlier observations (Retnakaran *et al.* 2001, 2003).

Previous studies have reported differences in the toxicity of tebufenozide and/or methoxyfenozide as a function of the larval instar examined. While in two cases the authors noted a reduction in potency with advancing developmental stages of the test species (Knight *et al.* 2001; Moulton *et al.* 2000), in two other cases the authors observed the very opposite trend (Sáenz-de-Cabezón Irigaray *et al.* 2005; Smagghe and Degheele 1994). It is important to point out, however, that these conclusions were based on a comparison of lethal concentration (LC) estimates as opposed to the lethal dose (LD) estimates reported here. To estimate LC_{50} values with lepidopteran larvae, either foliage or artificial diet is dipped into solutions of the test compound prepared at different concentrations. While this approach has the advantage of being more representative of a field situation and provides more relevant information for the selection of application rates, larvae submitted to such an assay will consume increasing amounts of food as they advance in development and will therefore acquire vastly different doses of the test compounds, which are typically not reported. Therefore, differences in feeding rates among the insect species examined in the aforementioned studies could, in part, explain some inconsistencies in the trends that were reported. For the present work, we opted for the estimation of lethal dose values because our intention was to select doses (LD_{50}) that would enable a valid comparison of differential gene expression among larvae of distinct developmental stages and exposed to different test compounds. However, a simple comparison of LD_{50} values between different developmental stages does not take into account developmental differences in the ability of larvae to metabolize and/or excrete the test compounds, which should be related, at least in part, to larval mass. Recognizing that the ratios reported in Table 1 are not an accurate reflection of the true relationship between size and metabolism, the lower LD_{50}/mass ratios calculated for 4th instars nonetheless point in the same direction as two independent studies that reported an age-dependent rise in susceptibility to tebufenozide (Smagghe and Degheele 1994) and methoxyfenozide (Sáenz-de-Cabezón Irigaray *et al.* 2005) on the basis of LC_{50} values. Here, however, the developmental difference in LD_{50}/mass ratio was substantially smaller for tebufenozide than for methoxyfenozide (Table 1), again indicating a difference in the development-dependent response to the test compounds.

In conclusion, methoxyfenozide was, overall, more potent than tebufenozide in causing mortality in *O. leucostigma* larvae. In addition, analysis of the present data suggests that susceptibility of later instars to tebufenozide is significantly compromised

while that to methoxyfenozide is less so, which indicates that the mechanism conferring resistance to tebufenozide in older larvae is either ineffective or less effective in the case of methoxyfenozide. Transcriptional analysis should help pinpoint what these differences may be at the gene expression level.

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PHYTOMYZA KRYGERI HERING (DIPTERA: AGROMYZIDAE), A NEW INVASIVE SPECIES FOR NORTH AMERICA ON COLUMBINE

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Abstract *J. ent. Soc. Ont.* 148: 13–21

The European *Phytomyza krygeri* Hering (Diptera: Agromyzidae) is recorded for the first time in North America, with specimens collected in Ottawa, Ontario. Additional records represented by immature stages and characteristic larval feeding damage from Massachusetts and Wisconsin require confirmation. Ottawa adults have been observed on European columbine (*Aquilegia vulgaris* L. [Ranunculaceae]), mostly on flowers where oviposition occurs, and larvae and puparia have been recovered from seed pods in which the larvae feed on seeds.

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Introduction

Over 450 species of Agromyzidae are recorded as occurring in Canada (Spencer 1969; Spencer & Steyskal 1986; Zlobin 1996; Boucher & Wheeler 2001; Boucher 2002, 2003, 2004, 2009, 2012a, 2012b; Lonsdale 2017), with nearly 40% of these belonging to *Phytomyza* Fallén, the most diverse genus of Agromyzidae. The actual number of species in this genus is much higher, however, with many undescribed species known to the author. A revision of this immense genus in Canada is under development, but advance publication of distributional records for one species in particular is here deemed necessary because it is both invasive and occurring on a commercially available ornamental plant: *Phytomyza krygeri* Hering, a seed-feeder on the introduced European columbine (Ranunculaceae: *Aquilegia vulgaris* L.), is here recorded for the first time in North America.

In the spring of 2015, the author noticed adults of an unknown species of Agromyzidae on cultivated European columbine at his home in Ottawa, Ontario. These adults were immediately collected as they differed strikingly in colour and size compared to other local Agromyzidae on this host. They aggregated and oviposited not on the leaves, but on the flowers, which are held quite high above the leafy base. Larvae and puparia were subsequently located in infested plants in the Ornamental Gardens at the nearby Central Experimental Farm. Specimens continue to be found at both of these locations in 2017. The identity of this species as *Phytomyza krygeri* was established upon the dissection and ex-

amination of the phallus (Figs 9, 10; also see Spencer (1976: figs 763, 764)), which is highly diagnostic. Larvae and puparia were also located in the developing seed pods (Fig. 3); some were reared to adulthood and some were preserved in ethanol. This species is photographed, diagnosed and discussed, and the male genitalia illustrated.

The known European distribution of *Phytomyza krygeri* is England (Spencer 1972), Germany (Hering 1949), Denmark (Spencer 1973), the Netherlands, the Czech Republic, Poland (Martinez 2017), Slovakia (Straka 2005), Norway, Finland (Spencer 1976) and Sweden (Spencer 1992). Confirmed Nearctic records are restricted to Ottawa, Ontario, although additional records requiring validation includes feeding damage and immatures of what is likely to be *P. krygeri* in Northfield, Massachusetts (C.S. Eiseman pers. comm.), and in Wisconsin at Battle Bluff Prairie State Natural Area, Vernon County (Linden 2017).

Since its host plant is widely cultivated as an ornamental, it likely entered North America via the nursery trade. Ornamental industry sales represent the largest horticultural sector in Canada, with sales of $2.02 billion in 2015 and the production of 11.7 million outdoor perennial plants domestically (Agriculture and Agri-Food Canada 2016). Global imports of live trees and plants, including roots and mushroom spawn into Canada is also immense, totalling nearly $178 million in 2017 (Statistics Canada 2017). With this volume of plant products being imported and circulated within Canada each year, it is not a question of whether non-native species will be introduced, but how frequently they will arrive, establish and spread.

Other recent invasive Agromyzidae in North America include the Palaearctic *Phytomyza petoei* Hering, reported by Boucher (2009) on cultivated *Mentha* L. (Lamiaceae, likely peppermint) in Montreal. The Palaearctic *P. gymnostoma* Loew, a pest on *Allium* L., has been detected on leeks in Pennsylvania (Pennsylvania Department of Agriculture, 2017). *Ophiomyia kwansonis* Sasakawa is a leaf miner on the ornamental daylily (*Hemerocallis* L. [Hemerocallidaceae]) that was accidentally imported from east Asia to the United States via commercial trading prior to 2008, and is likely now distributed throughout the lower 48 states (Williams and Steck 2014); it is expected to eventually invade Canada.

Materials and Methods

Adults, larvae and puparia were all collected into 95% ethanol, with adults subsequently moved to a 100% ethanol bath and critical point dried, followed by gluing on pinned paper points. Abdomens were prepared by maceration in hot Lactic Acid followed by washing in demineralized water; dissected abdomens are stored in glycerine in genitalia vials pinned with the specimen. Genitalia were illustrated on a slide in glycerine using a camera lucida attached to a Nikon Optiphot. Larvae and puparia were reared in Petri dishes with a moist paper towel; puparia were overwintered in a fridge for five months. All material is deposited in the Canadian National Collection of Insects, Arachnids & Nematodes (CNC). Terminology follows Lonsdale (2017). Illustrated structures are shown proportionately in size to each other, except for the epandrium, which is reduced.

FIGURES 1–4. *Phytomyza krygeri*, adult male: 1, dorsal; 2, left lateral. 3, Seed pod of *Aquilegia vulgaris* L., opened, with brown damaged seeds to left and *P. krygeri* puparium (dark brown) and larva (yellow, recovered from separate pod) to right. 4, Examples of seed pods affected by feeding of *P. krygeri* larvae, with unaffected sample at far right.

Material Examined

CANADA. **Ontario:** Ottawa, 695 Malibu Tr., 45°22′16.29″N 75°42′57″W, O. Lonsdale, adults on *Aquilegia*, 23.v.2015, CNC422989-422996 (5♂ 3♀), 10.v.2016, CNC586948-586952 (5♂), 27.vii.2017, CNC799449-799452 (1♂ 3♀), ex seed pod *Aquilegia*, 30.iv.2015, CNC457768 (6 larvae + puparia), ex seed pod *Aquilegia*, 30.vi.2015, em. 4-7.iii.2016, CNC507014-507046 (16♂ 17♀), ex seed pod *Aquilegia*, 20.vi.2017, CNC799655 (6 larvae + puparia), Ottawa, Central Experimental Farm, Ornamental Gardens, 45°23′21″N, 75°42′28″W, O. Lonsdale, ex seed pod *Aquilegia*, 30.iv.2015, CNC457769 (11 larvae + puparia) , ex seed pod *Aquilegia*, 2.vii.2015, em. 4-7.iii.2016, CNC507006-507013 (6♂ 2♀).

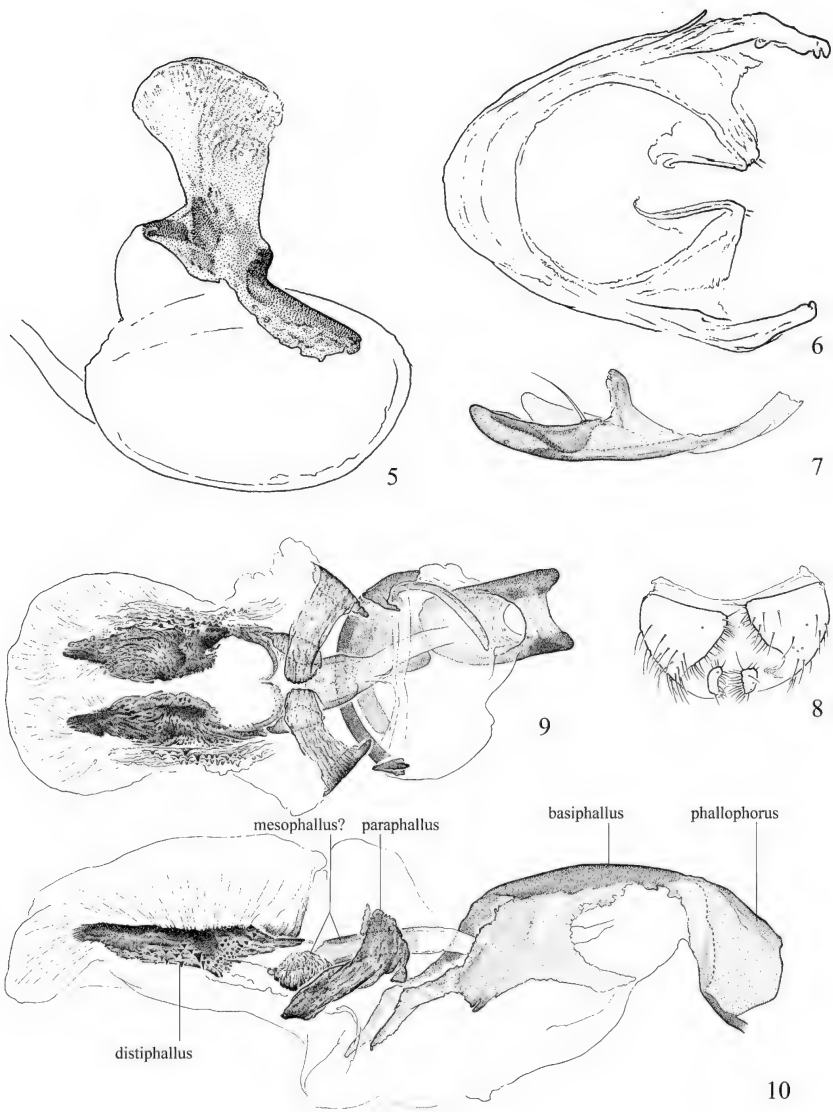
Species Diagnosis

The adult (Figs 1, 2) is predominantly dark brown to black with a slight greyish pruinosity that is denser on the dorsum of the thorax. The halter, the membrane between the sclerites, the apices of the femora, and most of the head are contrastingly yellowish-white to light yellow; the scape and pedicel are brownish, the first flagellomere and large ocellar spot are black, the back of the head is similarly dark, and the orbital plate is partially dark brownish-black posteriorly with dark spots at the base of the fronto-orbitals; the acrostichal setulae are very scattered and usually in approximately four rows at the level of the transverse suture, although there sometimes are as few as three or two; wing length is 2.2–3.0 mm. Typical of *Phytomyza*, *P. krygeri* also has the costa extending to vein R_{4+5} and the orbital setulae are proclinate (some erect), and like most other *Phytomyza*, cross-vein dm-cu is absent and the radial veins are inserted more basally on the costa.

Confident diagnosis of the species is best made via examination of the highly atypical male genitalia (Figs 5–10); while larger and greyer than other species on columbine, verification of genitalic structures is always recommended, especially to eliminate the possibility of misdiagnosis of other incidental *Phytomyza*. The plates of the basiphallus are wide, broadly fused dorsobasally, and apically extend as one pair of narrow, chevron-shaped processes. The paraphalli are wide, dark and striated. The homology of the apical components of the phallus are uncertain due to their highly derived appearance, but the mesophallus appears to form a pigmented and slightly widened apical section of the ejaculatory duct with one pair of striated dome-like accessory sclerites, and the distiphallus appears to be a heavily striated and minutely spinulose bifid plate with a large membranous dorsal fringe. The ejaculatory apodeme is small with a large, clear sperm sac.

Discussion

Phytomyza krygeri was described by E.M. Hering (1949) from 32 specimens, including one male and one female, each of which were treated as a "Type" (=syntypes); 30 specimens of unspecified sex were designated as paratypes, one examined female of



FIGURES 5–10. *Phytomyza krygeri*, male genitalia: 5, ejaculatory apodeme; 6, hypandrium, ventral; 7, postgonite, left lateral; 8, external components, ventral; 9, phallus, ventral; 10, phallus, left lateral.

which (ex "*Aquilegia*") is deposited in the CNC. The specimens were reared by J.P. Kryger from *Aquilegia* L. in Thoreby, Denmark, which on at least one specimen label is clarified as being the species *Aquilegia vulgaris* (Siedlar 1996). Specimens emerged from puparia from 10–13.iii.1948.

So far, this fly has only been reared from two cultivars of *Aquilegia vulgaris*, a perennial species in the family Ranunculaceae. The plant was introduced to North America as an ornamental and now occurs throughout much of Canada from Manitoba to Newfoundland and Labrador, and in much of the eastern United States including Iowa, Wisconsin, Illinois and Georgia, but also in the state of Washington (United States Department of Agriculture Natural Resources Conservation Service 2017). Host plants in Ottawa have not been found near native species of *Aquilegia*, which so far are not known to be attacked, but the fly's affinity for other host species should be more closely examined to discover its potential for spread to the native Nearctic flora. Until then, the fly's potential for damage in introduced regions can only be speculated upon.

Aquilegia species are mostly attacked by leaf-miners aside from the seed-feeding *Phytomyza krygeri*, the root-mining (and possibly stem-mining) *P. aquilegiophaga* Spencer [Canada] and the external stem-mining *Ophiomyia aquilegiana* Lundqvist [Europe]. These leaf-miners include the polyphagous *Liriomyza sativae* Blanchard [widespread] (Martinez 1993) and seven other *Phytomyza* species; of the *Phytomyza* species, *P. aquilegiana* Frost, *P. aquilegioides* Sehgal, *P. aquilegivora* Spencer, *P. columbinae* Sehgal and *P. sonorensis* Spencer are Nearctic, *P. aquilegiae* Hardy is European and *P. minuscula* Goureau (Spencer 1990) is Holarctic. Spencer (1990) suggested that these species (possibly referring to all *Aquilegia*-feeding *Phytomyza*) belonged to a single species group, but this will likely not stand up to scrutiny based on an overview of the male genitalia, even when the root-, stem- and seed-feeding species are excluded.

Spencer (1973, 1990) noted a similar species feeding in seed heads of columbine meadow-rue (*Thalictrum aquilegifolium* L. [Ranunculaceae]) in Switzerland, *Phytomyza thalictri* Escher- Kündig, which is very similar in genitalic morphology (Spencer, 1990: figs 189, 190) and the likely sister-species to *P. krygeri*. This host plant occurs in the Nearctic in Ontario and New York as an introduced species (United States Department of Agriculture Natural Resources Conservation Service 2017). There are also obvious similarities in morphology to *P. delphinivora* Spencer (Spencer 1990: figs 56, 57), which is known from Canadian *Delphinium* L. (Ranunculaceae).

In Ottawa, adult *Phytomyza krygeri* are seen in the spring on developing and fully formed flower-heads, which are suspended on long stalks above the rest of the leafy plant body. Specimens rest and fly around the flower heads and sometimes the stem and lower leafier parts; copulation occurs on the flower, and they return to the plant within one or two minutes if disturbed. Minute female feeding punctures made by the ovipositor may be seen on the petals, which grow and distort as the petals grow. Additional feeding punctures were sometimes seen on the leaves, but these may be attributable to *P. aquilegivora* Spencer, which was found leaf-mining on the same plant.

The larva (Fig. 3) is bright yellow, very motile, and described in detail morphologically by Dempewolf (2001). Formation of puparia (Fig. 3) is completed by the end of June, with no more than one puparium forming within a pod (the larva in Fig. 3 was taken

from a second pod and did not develop alongside the puparium shown). The larva may not be restricted to a single pod, however, as suggested by the presence of a large hole at the inner base of the pod that is about as wide as the larva and positioned opposite a similar hole in an adjacent pod. The puparium is small, subshining and dark brown, almost black; it is similar in size and appearance to the remains of the slightly paler brown seeds (Fig. 3) fed upon by the larva. These are in contrast to the untouched seeds, which are larger, fuller and shinier, with a hue that is bright green in the young seed and deep black in the mature seed. Adults emerged from puparia in early March after overwintering.

The affected seed pods are less robust than those unaffected, being distorted and discoloured from light to dark brown (Fig. 4). Seed pods affected early in development are more severely stunted and reduced in size. Damage to the host plant is less conspicuous than that of a leaf-miner and easily overlooked, although as a cultivated ornamental this may still cause impact on aesthetic value and subsequently commercial value. Furthermore, because the seeds are fed upon, larval damage reduces the reproductive potential of the plant.

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SPATIAL AND SEASONAL PATTERNS OF GROUND BEETLE (COLEOPTERA: CARABIDAE) ACTIVITY ACROSS HABITATS AT THE MONT ST. HILAIRE BIOSPHERE RESERVE, QUEBEC

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Abstract

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We investigated the seasonal variation and habitat preference of carabid beetles in mixed and hardwood stands, forest edge and meadow habitats at Mont St-Hilaire, southern Quebec, using pitfall traps from late May until the end of October 2007. We caught 1193 individuals belonging to 53 species of carabids. Among the 16 most abundant species, 5 were caught predominantly in early summer, 5 in mid-summer, and 5 showed a bimodal pattern of activity. Interestingly, there was no notable difference in terms of species composition between hardwood and mixed forests. However, ground beetle assemblages significantly differed between wet and mesic areas, and between forested and open or ecotonal areas. Fifteen indicator species were identified for different habitat types. Our results have implications for the conservation of carabid diversity, highlighting the importance of maintaining variation in canopy cover and dispersed hydric habitats in managed landscapes, but downplaying the importance of canopy species composition.

Résumé

A l'aide de pièges à fosse, nous avons déterminé la variation saisonnière et la préférence d'habitat des carabes de forêts mixtes et décidues, de bordures de forêts et de prés au mont St-Hilaire, QC, de fin mai à fin octobre 2007. Nous avons capturé 1193 individus représentant 53 espèces de carabes. Parmi les 16 espèces les plus abondantes, cinq furent capturées principalement au début de l'été, cinq en mi-été, et cinq ont montré une activité bimodale. Aucune différence n'a été observée dans la composition en espèces entre les forêts mixtes et décidues. Cependant, les assemblages de carabes différaient entre

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les zones humides et mésiques, et entre l'intérieur des zones boisées et les zones ouvertes ou les écotones. Nous avons identifié 15 espèces indicatrices des divers milieux. Nos résultats ont des implications pour la conservation de la diversité des carabes, soulignant l'importance de maintenir la variation dans le couvert forestier et les habitats hydriques dispersés dans les paysages aménagés, mais minimisant l'importance de la composition des espèces de la canopée.

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Introduction

Carabids are easy to sample and identify, and sensitive to environmental changes (Niemelä *et al.* 1996; Fournier and Loreau 1999; Antvogel and Bonn 2001). Accordingly, they are frequently used as indicators of biodiversity, ecosystem health, and the impacts of urbanization, forestry and agricultural practices (Pearce and Venier 2006; Work *et al.* 2008). However, despite a good grasp of their taxonomy and biology, gaps remain in our knowledge of both the phenology and habitat preferences of many species, impeding their use as indicators for management and conservation (Epstein and Kuhlman 1990). This problem is compounded by the considerable regional variability of carabid assemblages, which complicates comparisons between studies (Work *et al.* 2008).

Knowing the phenology of carabid beetles has scientific and practical benefits. It allows one to determine: the optimal sampling periods for each species (Werner and Raffa 2003); the overlap between the activity periods of carabids and agricultural pests, and therefore the suitability of the former as biocontrol agents (Fournier and Loreau 1999; Suenaga and Hamamura 2001); and the potential impact of invasive species (Barlow 1970; Loreau 1985; Niemelä *et al.* 1997). Regional surveys of carabid phenologies are needed because the foraging and breeding patterns that determine their seasonal activity are geographically variable (Goulet 1974; Thiele 1977; Loreau 1985; Bousquet 1986).

Habitat preferences of many carabids of eastern Canada remain poorly defined. Studies from different regions have shown relationships between carabid assemblages and soil moisture and pH (Germany; Antvogel and Bonn 2001), distance to forest edge (Germany; Sroka and Finch 2006), canopy cover (Hungary; Magura 2002), leaf litter composition and thickness (Finland; Koivula 1999), and understory, coarse woody debris and disturbance history (Western Canada: Work *et al.* 2004, 2010). However, geographically-specific information about the ecological distribution of local species is generally lacking, hampering the use of carabids as bioindicators and limiting our ability to predict the influence of environmental change.

In addition, the nature and extent of the ecological linkage between carabid communities and specific vegetation types is still debated (Niemelä *et al.* 1992; Antvogel and Bonn 2001). It has been suggested that specific carabid and plant assemblages tend to be associated because both respond to similar environmental factors, such as soil moisture and pH (Thiele 1977; Antvogel and Bonn 2001; Worthen and Merriman 2013). In contrast, vegetation structure and heterogeneity (though not taxonomic composition) may directly drive carabid assemblages (Brose 2003). The extent and the cause of variation in carabid

communities between forest types and along other habitat gradients are therefore still unclear.

Our study aims to (i) determine the phenology and habitat preferences of common species to facilitate their use as bio-indicators, and (ii), compare the species composition of carabid communities between mixed conifer and hardwood stands, hardwood stands and open habitats, on both mesic and hydric sites, in order to evaluate the relative roles of forest canopy and edaphic conditions.

Materials and Methods

Study sites

Sampling was conducted at the Gault Nature Reserve at Mont St-Hilaire, southern Québec, Canada (study sites located between 45°32'18" and 45°32'40" N, and between 73°09'25" and 73°09'45" W, altitude 160-240 m), in mixed and hardwood old-growth forest stands, in a meadow, and in a 15-20 year-old regenerating forest strip between the meadow and forest (hereafter called "ecotone"). The Gault Nature Reserve is a protected area of approximately 10 km^2 of primarily old-growth temperate forests. Dominant tree species in the hardwood forest stands are *Acer saccharum* Marshall (Sapindaceae), *Fagus grandifolia* Ehrh. (Fagaceae), and *Quercus rubra* L. (Fagaceae). The mixed forest stands are dominated by *Tsuga canadensis* L. Carrière (Pinaceae), *Pinus strobus* L. (Pinaceae), *A. saccharum*, and *F. grandifolia*, and is characterized by a needle-based litter and by a similar but generally sparser undergrowth than the hardwood forest stands. The 50-year-old meadow (formerly an orchard) is approximately 90 × 150 m in size, and is dominated by grasses and forbs (such as *Solidago* spp. L. (Asteraceae) and *Asclepias syriaca* L. (Apocynaceae)), with patches of *Rubus* spp. L. (Rosaceae). The ecotone is 5-10 m wide and was composed of young trees, particularly *Populus tremuloides* Michx. (Salicaceae) and *Rhus typhina* L. (Anacardiaceae), and grasses and forbs also found in the meadow. The forest areas include several streams (width 1-3 m) and marshes (smallest width >10 m).

Fifty-three pitfall traps were installed on 30 May 2007, as soon as all the snow had melted, within an area of about 0.5 km^2. Each trap consisted of a 750 ml container with a funnel constructed from acetate transparencies fastened inside. The funnel prevented the beetles from climbing or flying out of the trap. Traps were placed into the ground with their rim level with the ground surface. A roof consisting of a transparency (21.6 × 27.9 cm) was held 5 cm above the trap by four wooden dowels (secured using pins). The roof prevented rain and leaves from falling into the traps. In the forested sites, the leaf litter immediately surrounding the trap (within 20 cm) was removed to enhance catch rate and standardize the likelihood of catch between sites (a practice that follows Greenslade 1964; Liebherr and Mahar 1979). No preservative was used in the traps as samples were part of a joint study that required the beetles to be alive when collected.

We placed four to five traps per study site of 50 × 50 m. Within a site, traps were placed irregularly and separated from each other by at least ten meters. For each habitat type, we sampled at least two sites separated by over 300 m (except for the meadow and ecotone, which were too small; Table 1). A total of 18 traps were placed near (<1 m) areas

with surface water (9 traps near a stream, 9 traps near a marsh). Other traps were placed >10 m from water bodies.

Traps were checked 34 times between 30 May and 26 October 2007, every 2-7 days (4 days on average). Specimens were then killed in 70% alcohol, pinned and air-dried. Identifications were done using Lindroth (1961-1969) and voucher specimens at the Canadian National Collection of Insects, Arachnids and Nematodes (Agriculture and Agri-Food Canada), in Ottawa, where voucher specimens (CNC905809-CNC906032) of this study are kept.

Phenology

We described overall changes in the abundance of the 16 most abundant species (n>15 individuals) between early summer (30 May – 12 July), mid-summer (16 July – 8 August), late summer (13 August – 19 September), and fall (27 September – 26 October). Because of the variation in trap damage between sampling period (due to flooding or mammal activity), we adjusted the abundance of each species by dividing the observed number of individuals by the ratio of effective trap/days to total trap/days for each period. Patterns of seasonal activity were compared to other studies, particularly in light of the classic distinction between adult hibernators and larval hibernators (Lindroth 1945).

Species richness and composition across habitats

A multidimensional statistical approach was used to evaluate the relationship between carabid communities and site characteristics. Dissimilarity between traps was quantified using the Bray-Curtis measure of ecological distance (Bray and Curtis 1957). We conducted a Local Nonmetric Multidimensional Scaling ordination (LNMDS) using the function monoMDS from the vegan package (version 2.4-3) (Oksanen *et al.* 2017) in R (version 3.4.0; R Core Team 2017). LNMDS is considered a robust tool for indirect gradient analysis (Minchin 1987). NMDS is favored over other methods such as principal component analysis and "classical" Multidimensional Scaling because it does not assume a linear relationship between the dissimilarity measure and ecological distance, only monotonicity (Faith *et al.* 1987). The output of the ordination is a plot in two or three dimensions reflecting the ecological distance between the traps. With decreasing number of

TABLE 1: Number of traps and sampling sites for each habitat type.

	Hardwood forest		**Mixed forest**		**Ecotone**	**Meadow**	**Total**
Water proximity	>10 m	<1 m	>10 m	<1 m	>10 m	>10 m	
No. sites	3	2	2	2	1[1]	1	11
No. traps	13	10	10	8	9	4	54
No. effective trap-days	1663	1323	1269	1057	1090	516	6918

[1]The ecotone site was a continuous strip around the meadow.

dimensions, it becomes increasingly difficult for the graphical representation to accurately match the ecological distance between all the points. Therefore, the analysis includes a measure of stress, or "badness-of-fit", which is usually considered acceptable under 0.2 (McCune *et al.* 2002). The ordination was represented graphically using the R package ggplot2 (Wickham 2009), with 90% confidence ellipses drawn around the significant habitat clusters using the function stat_ellipse().

A PERMANOVA analysis was then conducted to test the significance of dissimilarity between the different habitat types (McArdle and Anderson 2001) using the adonis2() function from the vegan package in R (Oksanen *et al.* 2017), with 999 permutations. Furthermore, PERMANOVA pairwise comparisons were conducted for all factors. To minimize the risk of making a type-1 error from carrying out multiple pairwise tests on a single data set, we adjusted the p-values using the Bonferroni correction method (Rice 1989).

Habitat preferences

We used an indicator species analysis to test for species associated with the different habitats and groups of habitats (Fig. 1) (Dufrêne and Legendre 1997). This analysis returns an indicator value for each species ranging from 0 to 100% (with higher values representing a stronger association). The indicator value is a function of the species' fidelity to a habitat type and the species' specificity to that habitat type. Fidelity is the proportion of the individuals of a species that are found in a given habitat type, while specificity is the proportion of traps within that habitat type in which the species was present.

The indicator species analysis requires two inputs: the species abundance data and a partition of the sites into non-overlapping groups. To this end, we grouped traps first according to habitat, and additionally combined the habitats based on the results of the ordination and PERMANOVA analyses. Indicator species were therefore evaluated also for wet forest vs mesic forest vs open/ecotone sites, and for wet vs mesic sites (Fig. 1).

The analysis was performed in R using the indval() function of the labdsv package (Roberts 2016). We tested the statistical significance of the indicator value after 10,000 permutations and adjusted the p-value for multiple hypothesis testing using the function p.adjust() according to Benjamini and Hochberg (1995). Indicator species were retained when their indicator value (IV) exceeded 25%, following Dufrêne and Legendre (1997). If a species had a statistically significant association with more than one habitat group, we only indicated the group for which the species had the highest indicator value. Indicator values reported are rounded to the nearest percentage point.

Results

We collected 1,193 individual carabids belonging to 53 species. Of these, 6 species are non-native: *Carabus nemoralis* O.F. Müller , *Pterostichus melanarius* (Illiger), *Bembidion obtusum* Audinet-Serville, *Pterostichus vernalis* (Panzer), *Clivina fossor* (L.), and *Agonum muelleri* (Herbst) (Bousquet *et al.* 2013). A total of 12.4% of trap-days (8.6–14.8% among habitats) were lost due to damage by mammals or flooding by heavy rain.

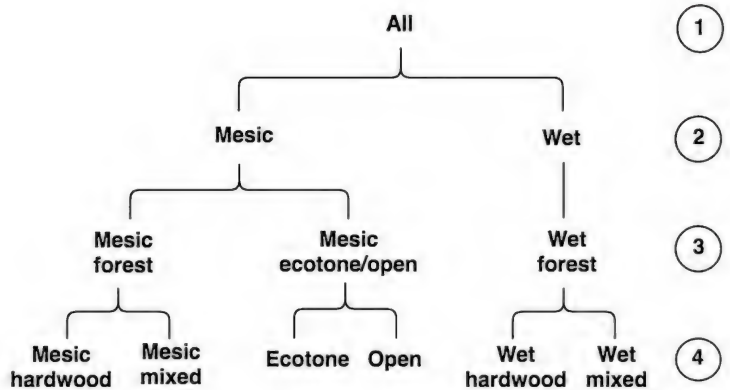

FIGURE 1. Habitat classification used for the indicator value analysis. Indicator values were calculated for each of the four levels of the classification.

Phenology

The overall trend was a decline of total carabid catches throughout the summer to late October. Among the 16 most abundant species, five species followed this general trend, having their peak of activity during early summer, such as *Agonum fidele* Casey, with 71% of individuals collected before 12 July (Fig. 2). Five species showed a unimodal pattern of activity, being rarely caught during early and late summer and peaking in mid-summer. Of these five species, *Pterostichus melanarius* and *Synuchus impunctatus* (Say) strongly show this pattern, with 68% and 73% of individuals collected during mid-summer, and peaking during the end of July, and early August, respectively. Another trend was a bimodal pattern of activity, shown by *Sphaeroderus lecontei* Dejean (38% collected in early summer and 44% in the fall), *Pterostichus pensylvanicus* LeConte (23% in early summer and 67% in the fall), *Pterostichus mutus* (Say) (65% in early summer and 24% in the fall), *Platynus decentis* (Say) (57% in early summer and 23% in the fall), and *Agonum melanarium* Dejean (59% in early summer and 26% in the fall).

Species richness and composition across habitats

For the LNMDS ordination, two dimensions produced a stress value of 0.17, which was deemed acceptable. In our preliminary analyses, several variations of the NMDS method were explored, including global and hybrid NMDS, as well as using log-transformed abundances. However, these approaches did not improve the stress level of the ordination solution and in some cases required three instead of two dimensions to remain below the threshold of 0.2. For this reason, we felt that the LNMDS method most accurately represented the true ecological distance between traps. Based on their carabid communities, the wet and mesic sites appeared to segregate from each other, and the open and ecotonal sites appeared to segregate from the forested sites. However, the hardwood and mixed traps were undifferentiated (Fig. 3).

Results from the PERMANOVA corroborated the patterns shown in the LNMDS ordination plot. Canopy type and moisture were statistically significant factors ($F_{3,47}$ = 3.3 and $F_{1,47}$ = 9 respectively, $p < 0.001$). However, when the open and ecotone sites were removed from the analysis, leaving only hardwood and mixed sites, canopy type had no effect ($F_{1,37}$ = 1.3, p = 0.2). Pairwise comparisons confirmed that there was no significant difference between the carabid communities in mesic hardwood and mesic mixed sites, or in wet hardwood and wet mixed sites ($F_{1,21}$ = 1.2 and $F_{1,16}$ = 1.5 respectively, p = 1). Furthermore, the difference between the ecotone and open traps was not statistically significant ($F_{1,11}$ = 2.3, p = 0.09), although the small number of open traps (n = 5) may partially explain this outcome. However, wet sites differed from mesic sites, and forested sites differed from the open and ecotone sites ($F_{1,52}$ = 7.8 and $F_{1,52}$ = 5.4 respectively, p = 0.01).

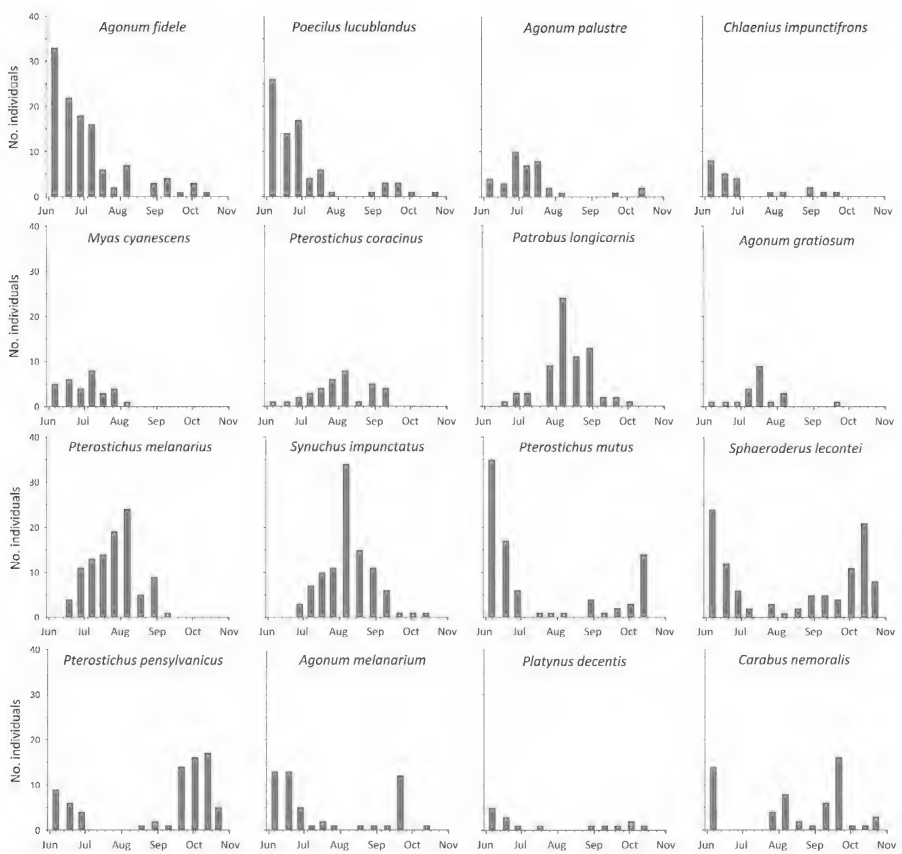

FIGURE 2. Seasonal activity of the 16 most abundant species of carabids collected at Mont Saint-Hilaire using pitfall traps (each bar represents an average of 494 ± 64 trap-days).

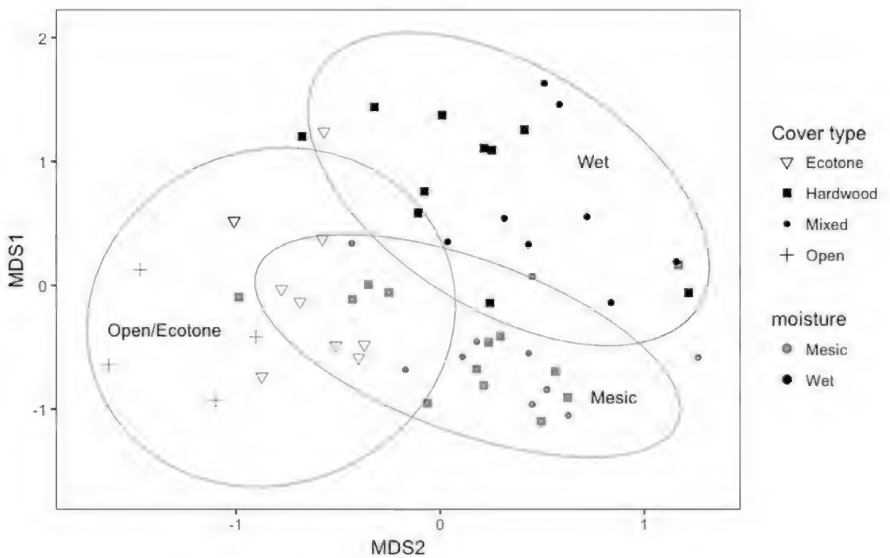

FIGURE 3. LNMDS ordination of the carabid community data by traps, showing 90% confidence ellipses around the three main habitat clusters: wet areas, mesic areas and open and ecotone areas (stress = 0.17).

Habitat preferences

Several indicator species were identified for each habitat type and habitat groupings (Fig. 4). *Synuchus impunctatus* was an indicator species for mesic areas (IV = 67, p = 0.005). *Pterostichus pensylvanicus* was the best indicator for mesic forest areas (IV = 64; p = 0.002), along with *Myas cyanescens* Dejean (IV = 48; p = 0.004).

The best indicator for wet areas was *A. fidele* (IV = 91; p = 0.002), and to a lesser extent *Chlaenius impunctifrons* Say (IV = 36; p = 0.017), *Bembidion frontale* (LeConte) (IV=35; p = 0.005) and *Agonum gratiosum* (Mannerheim) (IV = 34; p = 0.012). Wet hardwood forests were represented by *Patrobus longicornis* (Say) (IV = 57; p = 0.034) and *Bembidion graciliforme* Hayward (IV = 56; p = 0.029).

Several species were indicators for the ecotone and meadow together: *Poecilus lucublandus* (Say) (IV = 84; p = 0.002), *C. nemoralis* (IV = 75, p = 0.002), *P. melanarius* (IV = 65; p = 0.007), *Harpalus somnulentus* Dejean (IV = 39; p = 0.005), and *P. vernalis* (IV = 31; p = 0.014). *Agonum palustre* Goulet was an indicator for the ecotone specifically (IV = 86; p = 0.005), while *Amara lunicollis* Schiødte was an indicator for the meadow (IV = 65; p = 0.014).

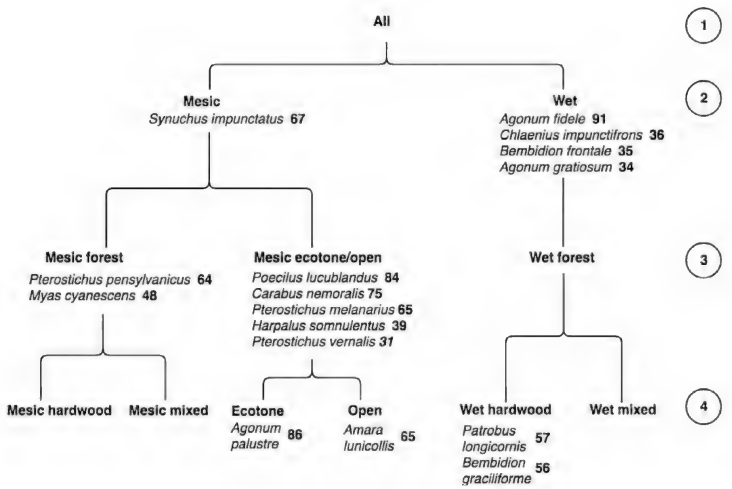

FIGURE 4. Dendrogram of habitats showing carabid indicator species for each partitioning level. Indicator values in bold. For p-values and species abundances, see Table 2.

Discussion

Phenology of common species

Platynus decentis, P. mutus, P. pensylvanicus, A. melanarium, and *S. lecontei* were active in spring/early summer but also displayed a significant peak of activity in autumn. These species hibernate as adults (Lindroth 1961-1969; Larochelle 1972; Goulet 1974; Bousquet and Pilon 1980; Bousquet 1986; Epstein and Kuhlman 1990; Larochelle and Larivière 2003), and oviposit in spring such that the emergence of a new generation of adults in autumn produces the second peak of activity (Larsson 1939; Larochelle and Larivière 2003).

Poecilus lucublandus is another adult hibernator (Kirk 1971; Bousquet 1986) for which an autumn activity period has been observed (Epstein and Kuhlman 1990), but we did not observe this pattern (79% caught before 12 July). Other spring-active species without an autumn activity period found in our study included *A. fidele*, *A. palustre*, *C. impunctifrons* and *M. cyanescens*.

Lastly, *S. impunctatus, Pterostichus coracinus* (Newman), *P. melanarius*, and *A. gratiosum* hibernate at least in part in the larval stage (Lindroth 1961-1969), which may explain the mid to late summer activity peak observed for these species, as seen in other studies (Lindroth 1961-1969; Bousquet 1986; Epstein and Kuhlman 1990; Werner and Raffa 2003).

Species composition and habitat preferences

Proximity to water. Proximity to water seemed to be more important than canopy

TABLE 2: Carabids collected in pitfall traps between 30 May and 26 October 2007. Indicator values showing habitat preferences, frequency (% of traps in which species was caught), and abundance (in parentheses) are indicated.

Species	IV[1]	Mesic forest		Ecotone (n=8)	Meadow (n=4)	Wet forest		Total caught
		Hardwood (n=13)[2]	Mixed (n=10)			Hardwood (n=10)	Mixed (n=8)	
Agonum fidele Casey	91**	15 (2)	20 (2)	25 (14)		**100 (56)**	**100 (42)**	116
Sphaeroderus lecontei Dejean		85 (44)	70 (22)	88 (15)	50 (2)	70 (11)	63 (10)	104
Pterostichus melanarius (Illiger)	65**	31 (30)	30 (7)	**88 (40)**	**75 (6)**	40 (16)	13 (1)	100
Synuchus impunctatus (Say)	67*	**77 (44)**	**80 (22)**	**88 (22)**	**50 (2)**	30 (6)	38 (4)	100
Pterostichus mutus (Say)		69 (23)	90 (31)	75 (15)		40 (10)	50 (6)	85
*Poecilus lucublandus (*Say)	84**	31 (7)	30 (4)	**100 (31)**	**75 (28)**	20 (5)	13 (2)	77
Pterostichus pensylvanicus LeConte	64**	**69 (20)**	**90 (44)**	13 (1)		40 (4)	50 (6)	75
Patrobus longicornis (Say)	57*		10 (1)	25 (4)		**70 (58)**	25 (6)	69
Carabus nemoralis O.F. Müller	75**	38 (8)	30 (7)	**88 (35)**	**75 (5)**	10 (1)		56
Agonum melanarium Dejean			20 (9)	25 (14)		50 (26)	13 (2)	51
Agonum palustre Goulet	85**	15 (2)		**100 (32)**		10 (1)	25 (3)	38
Pterostichus coracinus (Newman)		23 (13)	10 (1)			20 (8)	63 (13)	35
Myas cyanescens Dejean	48**	**62 (18)**	**50 (10)**	13 (2)	25 (1)			31
Chlaenius impunctifrons Say	36*	15 (2)		25 (4)		**60 (17)**		23
Agonum gratiosum (Mannerheim)	34*		10 (1)	13 (1)		**40 (15)**	**25 (4)**	21
Bembidion graciliforme Hayward	56*			13 (1)		**60 (15)**		16
Platynus decentis (Say)		15 (2)	20 (3)	13 (2)		50 (6)	38 (3)	16
Agonum retractum LeConte		23 (3)	20 (2)			10 (1)	25 (6)	12
Harpalus somnulentus Dejean	39*	8 (1)	10 (1)	**63 (6)**	**25 (1)**	20 (2)		11
Bembidion frontale (LeConte)	35**			13 (1)		**40 (6)**	**25 (2)**	9
Clivina fossor (L.)				38 (7)		10 (2)		9
Notiophilus aeneus (Herbst)		23 (3)	10 (1)	38 (5)				9
Pterostichus rostratus (Newman)		15 (2)	40 (6)			10 (1)		9
Pterostichus luctuosus (Dejean)					25 (1)	30 (6)	13 (1)	8
Pterostichus vernalis (Panzer)	31*			**25 (6)**	**50 (2)**			8
Amphasia interstitialis (Say)		8 (2)		25 (2)		20 (2)		6
Loricera pilicornis (Fabricius)			10 (1)			20 (4)	13 (1)	6
Platynus tenuicollis (Leconte)						10 (2)	25 (4)	6
Pterostichus tristis (Dejean)		15 (2)	20 (4)					6
Bembidion obtusum Audinet-Serville				25 (5)				5
Cymindis americanus Dejean		8 (1)	10 (1)	13 (1)	25 (1)		13 (1)	5
Elaphrus clairvillei Kirby			10 (1)			30 (4)		5
Amara lunicollis Schiødte	65**			13 (1)	**75 (3)**			4
Pterostichus patruelis (Dejean)						20 (4)		4
Gastrellarius honestus (Say)			30 (3)					3
Pterostichus corvinus (Dejean)						20 (3)		3
Pterostichus femoralis (Kirby)				25 (3)				3
Trechus apicalis Motschulsky							25 (3)	3
Acupalpus carus (LeConte)					25 (1)	10 (1)		2

TABLE 2 continued...

Species	IV[1]	Mesic forest		Ecotone	Meadow	Wet forest		Total
		Hardwood (n=13)[2]	Mixed (n=10)	(n=8)	(n=4)	Hardwood (n=10)	Mixed (n=8)	caught
Amara cupreolata Putzeys				13 (1)	25 (1)			2
Bradycellus kirbyi (G.H. Horn)				13 (1)			13 (1)	2
Chlaenius emarginatus Say				25 (2)				2
Olisthopus parmatus (Say)		8 (1)	10 (1)					2
Agonum muelleri (Herbst)				13 (1)				1
Amara angustata (Say)				13 (1)				1
Bembidion mimus Hayward							13 (1)	1
Bembidion muscicola Harward						10 (1)		1
Bradycellus semipubescens Lindroth				13 (1)				1
Calleida punctata LeConte				13 (1)				1
Notiobia nitidipennis (LeConte)				13 (1)				1
Pterostichus adoxus (Say)							13 (1)	1
Scaphinotus viduus (Dejean)			10 (1)					1
Agonoleptus conjunctus (Say)					25 (1)			1
Xestonotus lugubris (Dejean)				13 (1)				1
Total specimens		230	186	280	55	294	123	1168
Total species		21	25	35	14	30	23	53

[1] Only statistically significant indicator values above 25 are shown. Grey cells with bold numbers indicate the habitats represented by the species. Levels of significance: *: p<0.05, **: p<0.01

[2] Number of traps

composition or site openness, showing a stronger influence on carabid assemblages. Several species were shown to be associated with wet habitats: *A. fidele, P. longicornis, C. impunctifrons, A. gratiosum*, *B. frontale*, and *B. graciliforme*. These species have previously been found to occur primarily in moist areas (Canada/Alaska: Lindroth 1961-1969; Minnesota, USA: Epstein and Kuhlman 1990; Canada/USA: Larochelle and Larivière 2003). Conversely, three species, *S. impunctatus*, *P. pensylvanicus*, and *M. cyanescens* were consistently found away from water. The importance of moisture in determining the range of habitats used by different carabid species has been stated by others (Epstein and Kuhlman 1990; Niemelä *et al.* 1992), and the present data support this.

Habitat openness. Several species were significantly associated with open and semi-open areas: *P. melanarius, P. lucublandus, C. nemoralis, A. palustre*, *P. vernalis*, and *H. somnulentus*. Additionally, *A. lunicollis* was found to be correlated with fully open (meadow) areas. It is noteworthy that half of the introduced species we collected are indicators for the open and ecotone habitats, while the other half were also found mainly in this habitat but in too low abundance to obtain a significant indicator value.

Despite these general results, our experimental design did not permit an in-depth analysis of the carabid communities of open areas. We expect that a larger number of traps in meadow habitats would have revealed more indicator species for that ecosystem. In

addition, the association of *A. palustre* with the ecotone is probably an artifact, as many individuals were caught in a single trap located in an area with permanently saturated soil but far from any bodies of water. This species is known to be associated with wet habitats (Lindroth 1961-1969).

Nevertheless, we found that the carabid communities of mesic forest areas and of mesic open and ecotonal areas were more similar to each other than they were to those of wet forest areas. Sampling in wet open areas would be necessary to ascertain the relative importance of canopy cover and the presence of surface water; nonetheless, this result reinforces the finding that within forested areas, proximity to water is a major determinant of carabid community composition.

Forest canopy composition. Mixed and hardwood forests did not differ markedly in terms of the composition of their carabid communities. This supports findings of other studies indicating similar macroarthropod assemblages in hardwood forests compared with coniferous or mixed stands (Connecticut and Massachusetts, USA: Ellison *et al.* 2005; United Kingdom: Fuller *et al.* 2008; Virginia, USA: Rohr *et al.* 2009; Massachusetts, USA: Sackett *et al.* 2011).

Nevertheless, two species showed preferences between the two forest types: *P. longicornis*, and *B. graciliforme*, both indicators of wet hardwood stands. Additionally, *P. pensylvanicus* obtained a high indicator value for mixed stands (IV = 48; $p = 0.008$), though its maximal value was for dry forest stands overall. Curiously, a large-scale survey of carabids across northern Canada found that *P. pensylvanicus* occurs primarily in hardwood stands. Furthermore, this study found the carabid communities of boreal hardwood and mixed stands to be distinct in eastern Canada (Work *et al.* 2008). In contrast, our study agrees with other research in the temperate lowland forests of eastern United States and southeastern Canada that suggests that carabid communities do not differ significantly between forest types (Massachusetts: Sackett *et al.* 2011; Connecticut: Ingwell *et al.* 2012). It is possible that boreal forests differ from lower latitude forests in the distinctiveness of the carabid communities in hardwood vs. mixed stands, or that the geographic scale of a study influences the ability to detect these differences. Yet it generally appears that stand age, habitat heterogeneity, canopy cover, amount of dead wood and edaphic conditions are the key determinants of carabid communities, rather than the composition of the canopy (du Bus de Warnaffe and Lebrun 2004; Fuller *et al.* 2008; Bergeron *et al.* 2011; Worthen and Merriman 2013). The associations between carabid species and specific habitat types remains elusive, and although we feel confident about the differentiation of the habitats sampled into broad categories based on canopy openness and proximity to water, the finer ramifications of the indicator species analysis should be interpreted with caution.

It is apparent in this study that carabid communities differ more between wet and mesic habitats than they do between forest types, a divergence that was observed within stands. At a coarser scale, carabid and tree assemblages might both segregate along a gradient of dry to mesic to hydric sites, and thus a correlation between the two may become apparent (Bergeron *et al.* 2011; Worthen and Merriman 2013). However, this correlation would not necessarily indicate a causal relationship between taxa, but rather an independent, but parallel response to environmental variables.

Therefore, when evaluating the effect of canopy composition on carabid

communities, it is important to separate the effect of edaphic conditions. For example, some studies in the northeastern United States aim to predict the effect of shifts in canopy composition on carabid communities due to pest infestations like the hemlock woody adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae), but they do not account for edaphic conditions (e.g. Werner and Raffa 2000). Since hemlock-dominated stands tend to be more humid, we would suggest that this omission could lead to the potentially misleading conclusion that carabid communities are following gradients in canopy composition rather than moisture, as suggested by our data. Additional studies are needed to disentangle these two factors.

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A NEW FAMILY TO CANADA WITH THE DISCOVERY OF THE PLEASING LACEWING *NALLACHIUS AMERICANUS* (MCLACHLAN) (NEUROPTERA: DILARIDAE) AT THE OJIBWAY PRAIRIE COMPLEX IN WINDSOR, ONTARIO

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The pleasing lacewings (Neuroptera: Dilaridae) are a poorly studied and rarely collected group with seven species in the New World (Bowles *et al.* 2015). *Nallachius americanus* (McLachlan) is the only species in eastern North America and is currently known from 19 states (Bowles *et al.* 2015). The observation of a single male *Nallachius americanus* in an urban residential backyard immediately adjacent to Spring Garden Natural Area in Windsor, Ontario represents the first Canadian record of the family Dilaridae. Here

FIGURE 1. *Nallachius americanus* male photographed from Spring Garden Natural Area (Windsor, Ontario) on 15 July 2017.

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we review our observation and discuss the habitat from where it was collected including photo documentation.

The Spring Garden Natural Area (Spring Garden) is one of five closely associated natural blocks located in west Windsor, Ontario, collectively known as the Ojibway Prairie Complex. Spring Garden makes up approximately 117 hectares of the total 244 hectare complex and includes prairie, savannah and Carolinian species. Naturalized drains, meadow marshes and swamp forests also provide habitat for wetland species. The complex is most notable for its tremendous biodiversity in an urban landscape. A comprehensive terrestrial invertebrate study undertaken in the complex yielded 2217 species (Paiero *et al*. 2010). In addition a BioBlitz held in the Ojibway Prairie Complex in 2014 discovered 12 new invertebrate species to Canada, which is another testament to its vast biodiversity (Gardner-Costa *et al*. 2014).

On 15 July 2017, a 400W metal halide light and white cotton sheet were set up to casually observe and photograph the nocturnal insect diversity in Spring Garden Natural Area (42.261484° -83.048819°). No specimens were collected and only digital photographs were obtained. A single male *Nallachius americanus* specimen landed on the sheet at approximately 23:00 (Fig. 1). A voucher specimen was not collected, but several high quality digital macro photographs were taken to document the observation. S. Paiero (University of Guelph) confirmed the identification as *N. americanus*; it is the only species of pleasing lacewing known from eastern North America, leaving no concern of confusing it with other species. The male, distinguished from the female by its pectinate antennae (Carpenter 1947), was found in a residential backyard immediately adjacent to a mature oak

FIGURE 2. Sampling site and adjacent habitat where *Nallachius americanus* was photographed (Spring Garden Natural Area, Windsor, Ontario).

and hickory forested section (Fig. 2). The lower limbs of many of the mature oaks are dead and could provide microhabitat for *N. americanus* whose larvae develop under the bark and consume soft-bodied arthropods (Oswald 1998). The dominant groundcover nearest to the sighting is thick sensitive fern, *Onoclea sensibilis* L. (Onocleaceae). The leaf litter is thick and made up mostly of oak leaves, which persist largely unchanged all season long. The soil is deep, rich, fine and sandy.

This record is an example of how amateur natural historians can play an important role in identifying and recording their local biodiversity. The nature of our light trapping observations was casual and the encounter with *N. americanus* was strictly incidental. While photographing various taxa at the sheet, a small (~10 mm) moth-like insect with erect pectinate antennae and feathery wings caught our attention. The photos are high quality and are more than sufficient to record *N. americanus* in Ontario. No other attempts were made to locate this species. Steyskal (1944) collected 27 *N. americanus* specimens from nearby Detroit, Michigan; thus it is likely this species has been present in Canada all along, simply undetected, perhaps due to its apparent rarity and moth-like appearance. Since little is known about the extent of its range and ongoing occurrence in Ontario, future observations should be noted and a voucher specimen should be collected if practical.

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POSTER AND PRESENTATION ABSTRACTS ENTOMOLOGICAL SOCIETY OF ONTARIO ANNUAL GENERAL MEETING

University of Guelph, Guelph, Ontario, September 29 – October 1, 2017

J. ent. Soc. Ont. 148: 43–69

Plenary abstracts

Bee-ing serious about pollinators and food.

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Roughly one third of the food we eat depends on pollinators. Economically sustainable yields for 75% of global crops are byproducts of pollinator foraging activity (most importantly bees), moving between flowers collecting food and also carrying pollen to facilitate seed, fruit and nut production. Beyond crops, almost 90% of flowering plant species worldwide rely on animal-vectored pollination, making pollinators an essential part of natural ecosystem function and wider cultural values. Reports of global pollinator declines raise concerns for agricultural productivity, food security and reduced natural biodiversity. Pollinators are beautiful, fascinating, diverse and essential creatures that we cannot afford to lose.

Humans and human foods—gourmet meals for insects.

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The presentation will focus on common bed bugs, mosquitoes, and blow flies that use humans and human food, respectively, as "gourmet" meals. I will describe how we have identified the bed bug aggregation pheromone, and the experiments we have run in the laboratory and in bed bug-infested apartments to test the effect of synthetic pheromone as a trap lure. Shifting to mosquitoes, I will show that inflorescences of plants and human hosts provide similar cues that attract foraging mosquitoes, shedding light on the evolution of hemtophagy. Focusing on flies, it is well known that food soiled by regurgitate and feces of feeding flies is more attractive to flies then clean food. I will present the surprising mechanisms underlying this "fly factor" phenomenon. I will present experiments showing how this phenomenon can be used to develop effective trap baits.

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The root of the problem: why identity matters when dealing with *Delia* root-maggot flies.

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It is a general misconception, even among scientists, that insects of high economic relevance are well known and easy to identify. Using root-maggot flies as an example we will review how poor taxonomic knowledge and inappropriate sampling procedures can hamper the development of effective pest management strategies. New identification tools for the group will be presented and plans to optimize the monitoring and management of these major pests of vegetable crops in Canada will be outlined.

Poster abstracts

Dose responses to a range of pesticides by *Megachile rotundata*, a representative solitary bee.

Ansell, G.R.*, A.J. Frewin, A.E. Gradish, and C.D. Scott-Dupree.
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Solitary bees comprise a large portion of bee species, yet are poorly represented in the ecotoxicology literature. This limits our understanding of the effects of pesticides on solitary bees. Therefore, we aimed to generate additional baseline pesticide toxicity data for the alfalfa leafcutting bee (*Megachile rotundata* Fabricius) by determining the topical and oral LD50 of dimethoate, imidacloprid, permethrin, chlorantraniliprole, and captan for adult females.

Insect ID: using convolutional neural networks (CNNs) to photo identify insects on any mobile device.

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Advances in technology have made it possible to use convolutional neural networks (CNNs) to identify objects in photos almost instantly. Fields such as ornithology and botany have already taken advantage of this technology by developing mobile applications that allow any user to instantly identify birds and plants. However, there is no such app for entomological purposes. Insect ID is a University of Guelph student-operated project aiming to develop a mobile app for photo identifying insects using CNNs. We present our methods for building the app and explore future applications of CNNs for entomological purposes.

Culicids on the move: a population genetic analysis of *Aedes japonicus* japonicus (Diptera: Culicidae) in Newfoundland, Canada.

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Disease vector movement is central to understanding epidemiological risk. *Aedes japonicus japonicus* is an invasive mosquito species in North America. Since its detection in 1998, the species has spread to over 30 American states and most Canadian provinces. In its invasive range, *Ae. j. japonicus* is an aggressive biter linked to the transmission of important human pathogens. While movement into eastern Canada was predicted, its arrival in Newfoundland presents a more complicated picture with potentially significant boundaries to direct migration. Here I describe the outcomes of a study of the genetic relationships between *Ae. j. japonicus* populations in Newfoundland, and those of mainland Canada, and Europe.

The differing effects of landscape on the genetic differentiation and dispersal of the Rocky Mountain apollo butterfly *Parnassius smintheus.*

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Landscape variables that best explain genetic differentiation may not also best explain dispersal patterns, but many studies use genetic differentiation as a proxy for dispersal. I tested the effects of landscape on both genetic differentiation and dispersal in parallel, to explore the validity of using genetic differentiation as a substitute for dispersal data. I used circuit theory (Circuitscape) and least cost transect analysis to evaluate the effects of landscape on both movement and genetic differentiation of the butterfly, *Parnassius smintheus* in the Jumpingpound Ridge study system. I found genetic differentiation should be supplemented by other, more direct measures of dispersal.

The effect of environmental stressors on brown marmorated stink bug (H*alyomorpha halys*) overwintering physiology.

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In temperate North America, insects may encounter several environmental stressors - including low temperatures, desiccation, and energy drain – while overwintering. The Brown Marmorated Stink Bug (*Halyomorpha halys*) is an invasive pest that damages several economically important crops, thus threatening the Canadian agricultural landscape. Here, we aim to determine the relative importance of low temperatures, desiccation, and

energy drain in dictating *H. halys* overwintering success, and to determine if *H. halys* stress tolerance changes seasonally. We report the physiological responses exhibited by field-collected *H. halys* populations when exposed to environmental stress, and offer insight towards potential pest management strategies.

Freezing tolerance and the molecular mechanism in the Andean stick insect *Agathemera crassa* (Phasmatodea: Agathemeridae).

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Agathemera crassa inhabits the Andes Range at 2400 m a.s.l., where the minimum temperature reaches -6ºC. In this work we address how these insects respond to low temperatures at the physiological and molecular levels. We determined the supercooling point (SCP) and freezing survival of *A. crassa*. We found that all the tested organisms survived ice formation in their body fluids and that the measured parameters were not affected by collection season or cooling rate. Finally, we have detected potential antifreeze proteins and low molecular weight cryoprotective in hemolymph samples.

Specialist vs. generalist: which is the best pollinator? The reproductive biology and pollination ecology of the Nevada peavine (*Lathyrus lanszwertii*).

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Lathyrus lanszwertii Kellogg (Fabaceae) is an under-examined herb common to montane biomes in western North America. Work on *Osmia iridis* Cockerell and Titus (Megachilidae) indicates it specializes on *L. lanszwertii*, making the bee a potentially important pollinator. We investigated the reproductive biology of *L. lanszwertii* by excluding pollinators and hand pollinating flowers, and used visitor frequency to describe its pollinator ecology. Results indicate *L. lanszwertii* is self-compatible but requires insect pollinators to set seed. We also show *L. lanszwertii* is a generalist but is mostly visited by specialist foragers, supporting the hypothesis that *O. iridis* is an important pollinator.

Predacious diving beetle records from Akimiski Island, Nunavut (Coleoptera: Dytiscidae).

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The Dytiscidae (predacious diving beetles) are a large, diverse family of beetles, and are one of the most abundant aquatic beetle families found in freshwater habitats. We collected dytiscids from Akimiski Island (Nunavut), a large and remote island located in James Bay, 25 km off the coast of Attawapiskat, Ontario. We collected 151 specimens from six, helicopter-accessible ponds using aquatic bottle traps over an eight day period. This is a first report for the Dytiscidae fauna of Akimiski Island.

Queen bumble bee pollination networks across an urban-rural land use gradient.
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Habitat loss is one of the primary drivers of bumblebee (*Bombus*) declines. Many studies have investigated these impacts on bumblebee colonies; but, despite their fundamental role in colony establishment, few evaluate the impacts of habitat loss on bumblebee queens. We evaluate the floral hosts of 6 queen bumblebee species in urban and agricultural areas. We found that species richness increased with urban area; abundance decreased with agriculture; pollen richness increased with urbanization; and bumble-floral host nestedness decreased with agriculture. We conclude that bumblebee queens and foraging exhibit contrasting patterns with agricultural (-) and urban areas (+).

Relatedness among dominant, territory-holding males in a large carpenter bee, *Xylocopa virginica*: do related males compete?
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Male *Xylocopa virginica* compete for territories near nesting aggregations. Dominant males defend these territories to gain access to receptive females that frequent nests between foraging trips. The relatedness of dominant males should be higher than a random subset of males when fit mothers produce multiple fit sons. Conversely, when fit mothers only produce one son or less, regardless of their fitness, relatedness among dominant males should be quite low. Additionally, the kin avoidance hypothesis suggests that related males should actively avoid competition. In this case, related males should either disperse, or have proportionately less aggressive interactions than with non-relatives.

The effect of abiotic factors on carabid diversity and distribution in northern Ontario.
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Large-scale geographic distributions of carabids are governed by macro-scale climatic factors which are mainly driven by temperature. We examined several other hypotheses that might affect carabid diversity and distribution spanning 451,808 km^2 of Ontario's boreal forest. We tested hypotheses of temperature, continentality, precipitation, elevation, and watershed. We found that species composition differed by watershed but not by the other abiotic factors. Our results suggest that the dispersal of many species of Carabidae is enhanced by some mechanism operating at the watershed level, for example downstream movement of flotsam.

***Megachile rotundata*: a potential model for non-*Apis* bee risk assessment.**
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Currently pesticide risk assessments are based exclusively on data from the European honey bee (*Apis mellifera*), and these data are used to estimate risk to non-*Apis* bees. Yet due to differences in their biology, honey bee data may not accurately estimate the risk posed to non-*Apis* bees. As a managed pollinator with a well-understood biology, the alfalfa leafcutter bee (*Megachile rotundata*) is a potential model solitary bee for toxicological experiments; however, standardized methods for use with *M. rotundata* are lacking. We will present results from tier I (laboratory) and tier II (semi-field) method development experiments with *M. rotundata*.

Effects of introduced honey bees on native stem-nesting bees (Hymenoptera: Megachilidae) in temperate mixed-wood deciduous forests.
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A much-debated question is whether honey bees *(Apis melifera*), introduced into the New World, are influencing declines in native pollinators. While the role of competition in understanding declines is difficult to determine in mobile insects such as bees, introduced species could increase competition over resources and nesting habitat for native species. Thus, if honey bees are abundant, they are likely to outcompete native wild bees for both habitat and food resources. Here we investigate whether Eurasian honey bees influence the functional diversity and reproductive ability of native stem-nesting bees in South-Central Ontario.

New record and range extensions of bumble bees (*Bombus* spp.) in previously unsampled areas of northern Ontario and Akimiski Island, Nunavut.
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Northern Ontario (NO) will experience agricultural development and resource extraction, yet its biodiversity is undocumented. Bumblebees (*Bombus* spp.) are facing threats (land-use and climate change, competition, pathogen spillover) globally. Limited data from NO prevents the prediction of population changes over time. We collected 220 specimens during large-scale biodiversity surveys of NO and Akimiski Island (AI), Nunavut from 2009-2015. Trap methods included: Malaise, pan, Nzi, and pitfall. We report on 11 bumblebee species - NO, 5 - AI, 1 new species for Nunavut, 1 eastern and 1 northward range expansion, and the occurrence of 1 species of special concern in NO.

Efficacy of insecticides for management of brown marmorated stink bug (*Halyomorpha halys* Stål) in Ontario.

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Brown marmorated stink bug (BMSB), *Halyomorpha halys* Stål, is an invasive pest native to East Asia. BMSB is highly polyphagous and feeds on many economically-important fruit, vegetables, row crops, and ornamentals grown in Ontario. Therefore, effective pest management options for BMSB must be identified to prevent crop damage and significant economic loss for Ontario growers. We evaluated the efficacy of some insecticides for BMSB management by determining their residual contact toxicity to nymphs. Ten insecticides were tested using leaf-dip bioassays. Recommendations for BMSB management in Ontario based on our results will be discussed.

Soy-bees: targeting soybean field hedgerows for pollinator conservation may lead to increases in yield and biodiversity.

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Wild pollinator conservation is largely dependent on the increase of quality and quantity of habitat. Agricultural areas are good candidates for increasing biodiverse hedgerows as they frequently support large monocultures of crops. Previously, it was thought that self-pollinating crops, such as soy, do not benefit from the presence of pollinators. However, recent literature suggests that soy can benefit from insect pollinators in the form of increased yield, thus increasing profitability of the crop. In conventional soy fields, some species of wild pollinator have been observed suggesting that targeting these hedgerows could result in an increase of pollinators within soy fields.

Species-specific responses of pest flies to repellents applied on livestock animals.
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Pest fly species location on host animal influences efficacy of control methods. The presence of horn, face, stable and house flies on body parts and specific responses to several repellents and carrier products were studied on pastured dairy animals. Percent repellence with horn flies, located mainly on back, sides and belly, was higher than with any other fly species. Face flies were present on most body parts, and more difficult to repel. Repellence of the stable flies on legs was low. House flies were less common on animals in pasture. Species and location differences pose a challenge for repellent development.

Putting monarchs on the map: Using spatial analysis to inform habitat creation efforts.
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The eastern migratory population of the monarch butterfly (*Danaus plexippus*) has suffered a population decline of >80% over the last two decades. The monarch's widespread and multi-generational migratory route across North America poses a considerable challenge to conservation efforts. A spatially informed approach can identify those areas where conservation investments are most likely to impact monarch populations by identifying areas of monarch occurrence; existing habitat and potential connectivity between these; and landscape level pressures from human land use. Pollinator Partnership Canada is proposing to undertake a spatial analysis to plan monarch conservation efforts in Ontario. These results will help target geographic areas integrate monarch conservation efforts into ongoing habitat protection and recovery, and other species-at-risks actions.

Landscape composition and configuration affect genetic differentiation in the alpine butterfly *Parnassius smintheus*.
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Landscape composition and configuration are important in determining the spatial genetic structure of populations, as they may facilitate or inhibit dispersal and consequently gene flow. I use RADseq protocols to identify genetic differentiation among populations of the alpine butterfly *Parnassius smintheus* in western Alberta. I compare patterns of genetic differentiation to landscape parameters to identify which landscape features are important in shaping population genetic structure. Understanding how these populations are genetically structured, and how population structure is affected by the landscape, is critical to predicting their persistence and ability to adapt to a changing environment.

Preliminary steps to understanding the decline of native bumble bee (*Bombus* spp.) pollinators in Canada using citizen science data.

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Few investigations into extinction risk or reasons for decline have been completed in Canada for bumble bees (*Bombus*). We are using data from the citizen science program, BumbleBeeWatch (BBW), a database of *Bombus* records from the mid-1800s, and our own field surveys to answer: 1. Does BBW show increased distribution and abundance of *Bombus* as compared to researcher collected data? 2. How can BBW data increase status assessments and effect conservation actions? 3. Is habitat loss, urbanization, and/or agriculture driving declines? Proposed methods include calculations of biodiversity metrics, modelling of landscape changes and probability occurrences, and assessments of conservation status.

The effect of dog-strangling vine invasion on bee diversity and abundance along an invasion gradient in Rouge Park.

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Invasive plants pose a threat to bee populations by changing the native plant community structure. Dog-Strangling Vine is an invasive plant in Rouge Park and to evaluate its effect on bee communities, bee diversity and abundance were measured along an invasion gradient in 8 meadow sites from May to September 2016. A positive relationship was found between Dog-Strangling Vine and both bee abundance and diversity. A relationship was also identified between bee diversity other site characteristics, such as distance to agricultural fields and to the city, suggesting surrounding landscape characteristics may influence the structure of bee communities visiting plant communities.

Is molecular evolution faster in the tropics?

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Do insects inhabiting tropical environments evolve at faster rates than insects inhabiting temperate environments? While previous studies have focused on vertebrates, we provide an analysis of molecular rate differences using COI barcode sequences spanning all of Insecta. Using a novel bioinformatics tool, we discovered over 6590 latitudinally-separated species pairs, revealing a near-even pattern whereby rates of molecular are higher in the tropical species in just over half (51.4%) of the paired lineages. These results suggest that molecular rate differences may only play only a weak role in explaining the latitudinal diversity gradient and that standard mitochondrial molecular clocks may generally apply across latitudes.

Impact of invasive dog-strangling vine on urban plant-pollinator networks.

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Vincetoxicum rossicum (dog-strangling vine) has had devastating effects on North America, outcompeting native plant species, turning the landscape into a monoculture. Little is known about its impacts on native bee foraging practices. By analyzing pollen on bees caught in dog-strangling vine invaded sites, we aim to determine how bee diversity is being affected along an invasion gradient. Bipartite analyses show a significant decrease in plant-pollinator interactions with increasing invasion, and linear regressions show a marginally significant decrease in floral richness. These findings stress the urgent need to develop conservation plans specific to wild pollinators in areas threatened by invasive plants.

Toxicity of three plant compounds to the parasitic mite *Varroa destructor* and their selectivity for honey bee (*Apis mellifera*) workers and larvae.

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This study examined the toxicity of anethole and the essential oils of *Cymbopogon citratus* and *Tagetes lucida* to *Varroa destructor* and to honey bees under laboratory conditions. Anethole was the most toxic compound to mites (LC_{50}: 304.9 µg/ml), whereas *Tagetes* oil was the least toxic (LC_{50}: 1256.27 µg/ml). The most and least toxic compounds to worker bees were anethole and *Tagetes* oil with LD_{50}s of 35942 and 85381 µg/ml, respectively.

The expression of *AChE*, a gene that regulates the production of acetyl cholinesterase, was not altered in bees treated with the plant compounds. This study showed that anethole and *Cymbopogon* oil have potential for controlling varroa mites.

Brown marmorated stink bug distribution and phenology in Ontario.
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The brown marmorated stink bug (BMSB) (*Halyomorpha halys* (Stål)) is an invasive insect pest from East Asia that is established in Ontario, Quebec, and British Columbia. BMSB's mobility and highly polyphagous nature make it capable of causing significant economic damage to fruit, vegetables and field crops. To elucidate how many generations of BMSB are in Ontario, field-collected females were assessed for reproductive status using a 5-stage ranking system based on ovary development. Results from 2016 indicate that there is not a 2nd generation of BMSB in Ontario. Data from 2017 is still being analyzed.

Demographic and genetic effects on social spacing: lessons from fruit flies.
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The environment can limit the social space of individuals within populations, yet precise factors that influence social spacing are not well understood. We measured nearest-neighbour distance as a function of genotype, group size and kinship in small populations of *Drosophila melanogaster*. After accounting for genotype and population density *per se*, we found that flies will actively under-disperse, and that this behavioural 'clustering' was most pronounced when kinship was lowest and highest (the equivalent of outbred and inbred groups). Our results suggest that social spacing, even in a non-social insect, is sensitive to kinship, despite the absence of other environmental cues.

Can you have it all? Assessing links between plastic growth and reproductive traits in *Latrodectus hasselti* males.
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Latrodectus hasselti (redback spiders) exhibit developmental plasticity--final instar males alter development in the presence of rival males. We studied whether effects of diet and male density on development led to trade-offs with reproductive traits (mate searching and sperm investment). We found that males on high diets developed faster and were larger, and the acceleration of development was strongest in the presence of numerous competitors. Diet decreased endurance, but had no effect on sperm production. Our results indicate that diet affects development, that rival density may also be important, but there is no evidence

of trade-offs with sperm and endurance.

You are what your mother eats: maternal rearing condition impacts future larval growth trajectory in Asian longhorned beetle.

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The impacts of artificial rearing conditions on insect physiology are often under appreciated. Asian longhorned beetles (ALB) are a high risk invasive species in North America. We sought to streamline laboratory rearing protocols of ALB by providing artificial egg laying substrate instead of fresh log bolts. However, larvae from these two protocols differed significantly in weight, despite unlimited larval food availability following hatching. These results suggest an unrecognized impact of maternal rearing condition on long term larval growth in ALB and are an important consideration for insect rearing facilities and the long-term health of managed ALB colonies.

The effect of vertical stratification and forest management practices on spider (Araneae) communities in temperate forests.

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The canopy and forest floor have different physical structures and may cause arthropods to assemble themselves vertically. This stratification has implications for forest management, since long-term logging practices have altered the composition and physical structure of forests. We aim to describe spider (Araneae) community structure in two different heights and to understand community level responses to forest management. The community structures of spiders differed between heights but not between forest management sites, demonstrating that spiders may assemble themselves vertically. These results may have implications for conservation efforts, as canopy-specific arthropods tend to have a higher conservation priority.

Energetics of overwintering in the soil in the western bean cutworm, *Striacosta albicosta*.

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Overwintering insects risk depleting finite energy stores at elevated temperatures due to an increase in metabolic rate. However, insects can conserve energy behaviourally by selecting a microhabitat that buffers against high temperatures, or physiologically through metabolic

plasticity. The western bean cutworm (*Striacosta albicosta*) might use both strategies; the soil depth of prepupal chambers is a determinant of overwintering temperatures, and warmer conditions induce a decline in the thermal sensitivity of metabolism in the lab. Here, I discuss factors impacting the depth of overwintering sites, and test if metabolic plasticity or overwintering depth reduce winter energy drain.

Sampling Syrphidae using Malaise and Nzi traps on Akimiski Island, Nunavut.

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Syrphids are important dipteran pollinators of many plant species, for which the efficiency of certain trap types are unknown. We compared two methods for sampling syrphids: Malaise and Nzi traps. We placed traps on Akimiski Island, Nunavut, each summer, from 2008 to 2016. Overall, we collected 346 specimens of 51 species; 35 are new records to Nunavut. Malaise trapping caught more individuals and species than Nzi traps. More species were unique to Malaise traps as well. Our study is the first to quantify the trapping biases of Nzi traps for Syrphidae, a trap originally designed for biting flies.

Assessing the potential of common cover crops to provide seasonal forage to managed and wild bees in Southern Ontario.

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Floral resources installed within or around crops in can provide bees with additional and alternative forage sources in agricultural landscapes where floral richness can be at a deficit. Growers commonly use cover crops to manage weeds, improve soil health and fertility, and manage water. Support for pollinators is likely an added benefit of cover crops already in use. We examined the plant-pollinator interactions exhibited on seven commonly planted cover crops in order to outline the potential set of biodiversity benefits provided throughout the season.

Significant preference patterns for certain species of cover crop were exhibited by honey bees and the native bees. Borage attracted the greatest number of bee types (highest richness) ($p>0.05$). Alfalfa, buckwheat, daikon, and red clover were equally supportive of native bee richness; native bee abundance was highest across alfalfa, borage, buckwheat and daikon. Buckwheat attracted the highest abundance of bees in total ($p>0.05$). Honey bees showed a significant preference for borage and buckwheat above all other crops tested ($p>0.05$). We recommend that growers allow the forage to grow at least into July, and suggest that they are planted in areas that will not be used in for cropping, allowing for full season food

availability.

Presentation abstracts

What effect does temperature have on the body length of Neotropical Staphylinidae?
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The "counter-Bergmann Rule" (CBR) predicts that ectotherm body length should decrease at higher elevations (and lower temperatures) to permit animals to reach sexual maturity earlier (i.e. smaller) in sub-optimal conditions. We report on our test of the CBR using neotropical rove beetles (Staphylinidae: Coleoptera) from an elevation gradient in north-western Costa Rica. We measured total body length using focus-stack specimen images. We found no support at the family level for the CBR, indeed the subfamily Aleocharinae displayed a trend opposite to what we predicted. We suggest that research to test the CBR should occur at fine taxonomic scales (genus or species).

Investigating queen influence on worker behaviour using comparisons of queenless and queenright workers.
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Eusocial sweat bees are capable of behaving as queens or workers. Few females become queens, and those that do manipulate the reproductive behaviour of other females in the nest. We collected *Lasioglossum laevissimum* workers from queenright and queenless nests to understand how worker behaviour changes without a queen. We also compared social characteristics of *Dialictus* species to understand how queens of closely related species manipulate workers. Queenright *L. laevissimum* workers were just as likely to have developed ovaries as queenless workers, which means that continuous manipulation by the queen plays a minor role in influencing the reproductive behaviour of workers.

Are soldiers a source of genetic novelty in termites?
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The termite soldier caste has pronounced features adapted to colony defence. In this study, we exploit RNA-Sequence data to show that genes with soldier-biased expression from the Eastern subterranean termite *Reticulitermes flavipes* are more taxonomically restricted on the tree of life than are genes with no caste-biased expression. This pattern suggests that

selection has favored novel gene recruitment during termite soldier evolution, potentially from non-coding regions of the genome. Given that *Reticulitermes* and most other termite soldiers are sterile, any past selection for genetic novelty of this caste must have been indirect and mediated through reproducing relatives.

The effects of stand diversification on neotropical beetle assemblages.

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Sustainable agroforestry as a means of reforestation and ecosystem stabilization has gained a lot of attention. However, herbivorous insects can jeopardize the establishment and sustainability of such plantations. We report on beetle communities in the Sardinilla plantation, in terms of dominant species and functional groups. We also test the effect of stand diversification on beetle and functional diversity and structure as well as the effects of key local environmental variables on beetle communities in the context of ecosystem functioning. Overall, stand diversification promoted high functional diversity and resilience to the ecosystem. Also, variables affecting microclimate drove beetle community assemblages.

Assessing dimethoate as a toxic standard for use in semi-field pesticide toxicity studies with *Bombus impatiens* (Cresson).

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Regulatory agencies soon will require agro-chemical companies to provide toxicity data on bumble bees for pesticide registration and re-registration, and thus risk assessment protocols for bumble bees are needed. The purpose of this experiment was to determine the suitability of dimethoate as a toxic reference standard for use in regulatory pesticide risk assessments with *Bombus impatiens* Cresson. Forty screened tents were placed over flowering buckwheat plots. Plots were sprayed with dimethoate at 40 g a.i./h, 80 g a.i./h, or 200 g a.i./h. Controls were sprayed with water. Results of forager assessments and development endpoints will be presented and discussed.

Establishing and observing the squash bee (*Peponapis pruinosa*) in captivity.

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The native squash bee (*Peponapis pruinosa*) is a well-known wild pollinator of pumpkin,

squash, and zucchini crops (*Cucurbita* spp.) on farms in Ontario. However, this bee has not previously been studied under controlled conditions and observations of nesting and foraging behaviour are very limited. We established captive squash bee populations in hoophouses to study their nesting, foraging, and flower handling behaviour as part of a larger study to determine the impacts of insecticide exposure on squash bee health. Here we describe the process and pitfalls of establishing a captive population and present preliminary data describing nesting and foraging behaviour.

Structural characterization of RdRp and pseudoknot (PK) I of Israeli acute paralysis virus isolated in China.

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To understand the diversity and pathogenesis among the different Chinese isolated IAPV, the Beijing IAPV (IAPV-BJ) strain was isolated and purified from diseased honey bee samples. Phylogenetic comparison on RNS-dependent RNA polymerase (RdRp) region at the amino acid level results indicated its genotype was closer to Chinese and Korean strains. While the structure model of intergenic region-internal ribosome entry site region suggested that is possessed similar stem-loop and pseudoknots (PK) structure, the PK I was strikingly distinct from those of other known strains. These results demonstrate the reason why different original strains have significant difference in virulence.

Rove beetle diversity and community structure across a neotropical elevation gradient.

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As elevation increases, climatic factors can become physically stressful for inhabitants, changing community structure and composition. These environmental stressors impose an environmental filter on communities and can result in species being more specialized and less diverse. We focus our research on examining this in one of the most abundant leaf litter fauna, the rove beetles (Coleoptera: Staphylinidae) across an elevation gradient in Costa Rica. We use DNA barcodes and classic taxonomy to quantify diversity and phylogenetic community structure. Preliminary results suggest that diversity increases with elevation, and that communities are more related at both higher and lower, more environmentally stressful, elevations.

Distribution, abundance and biological control potential of the swede midge parasitoid, *Synopeas myles*.

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The swede midge, *Contarinia nasturtii* (Diptera: Cecidomyiidae) is a pest of *Brassica.* The parasitoid, *Synopeas myles* (Hymenoptera: Platygastridae), was found in southern Ontario in 2016 and identified by morphological and molecular methods. Distribution and abundance of *S. myles* in Ontario canola agroecosystems was determined through collection of infested plants throughout the 2016 and 2017 growing seasons. Samples were monitored for parasitoid and swede midge emergence and parasitism rates determined for each site.

Looking deeper into pollinator food webs using DNA barcoding: applications for the agri-environment.

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DNA barcoding technology can be used to identify the plant species from just the DNA within the pollen grains. We used this technology to investigate pollinator foraging networks in the urban environment by taking pollen from the insects' bodies and extracting the DNA (not harming the pollinator). This was used to inform urban pollinator planting for pollinators. These networks provide a lot more detail than observation alone and could be used to inform pollinator agri-environment schemes and flowering crop planting in general.

RNA interference of worker sterility genes: testing mechanisms of reproductive regulation in honey bees (*Apis mellifera*).

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Honey bee (*Apis mellifera*) workers are well known for their selfless tendency to forego reproduction in favour of directing help towards their queen mother. In this study, we use RNAi-interference to knock-down single genes previously identified as important to regulating this altruistic response to queen pheromone. This summer we used field trails to knock-down *fruitless* and monitor changes to worker behaviour and gene expression. Our results are unfolding but initial results suggest that workers do respond to the RNAi treatment at this and potentially other loci. We interpret our initial results in a broader

context of honey bee sociobiology.

Niche or neutral? Wetland permanence influences aquatic macroinvertebrate diversity and community assembly.
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Aquatic macroinvertebrates in non-permanent wetlands have developed desiccation strategies to survive drawdown. These communities must reassemble each year and thus provide a unique opportunity to explore diversity patterns along a gradient of hydroperiod and implications for community assembly. We observed that alpha and gamma diversity increased with wetland hydroperiod as more taxa can colonize and persist. Beta diversity was highest in temporary wetlands, suggesting high taxonomic turnover and that stochastic dispersal processes are initially important in shaping aquatic macroinvertebrate communities in wetlands. However, in permanent wetlands, established communities persist year-round and can eventually reach a more homogenous community state.

InscalisTM insecticide: a novel insecticide for management of piercing-sucking pests.
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*[1]BASF Canada Inc., 100 Milverton Drive, 5th Floor, Mississauga, Ontario, L5R 4H1, Canada, [2]BASF Corporation, 26 Davis Drive, 27709-3528 Research Triangle Park, 27709, United States; *Jessica.rush@basf.com.*

Inscalis™ insecticide is an innovation from BASF for control of piercing-sucking pests across a broad range of crops. Representing a unique chemical class, this new insecticide offers a resistance management option for control of devastating insects such as aphids, whiteflies, and certain leafhoppers, psyllids, and scales. A favorable environmental profile, control of pests resistant to other insecticides, and low acute toxicity to pollinators and beneficial arthropods are a few of the key benefits of Inscalis insecticide. These features highlight the value of this new insecticide within resistance management and integrated pest management programs.

Effect of *Varroa destructor* homogenate and parasitism on haemocyte concentrations and gene expression in honey bees.
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Circulating haemocytes are responsible for defensive and healing mechanisms in the honey bee, *Apis mellifera*. *Varroa destructor* parasitism and varroa homogenate injection showed similar reductions in total haemocyte concentrations in bees. Honey bees with different

haemocyte concentrations were compared for the expression patterns of hemolectin, prophenol oxidase, and class C scavenger receptor. The expression of *AmPpo* correlated well with haemocyte counts indicating that melanisation is associated with those responses. Thus, expression of *AmPpo* might be a suitable biomarker for haemocyte counts as part of cellular defenses against mite compounds, varroa parasitism and perhaps other conditions involving healing and immunity.

Identifying and synthesizing pheromones of the *Anopheles* genus to be used in controlling the spread of Malaria.

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The *Anopheles* genus of mosquito remains one of the most prolific disease carrying vectors and caused more than 212 million deaths in 2015 alone (WHO). Many ways to control the spread of malaria have been proposed and one such way is using semiochemicals. Semiochemicals that the Anopheles genus use to communicate can be identified, synthesized and subsequently put in pheromone traps to control the population. To find these novel semiochemicals structure, analytical and spectroscopic techniques such as GC-MS, NMR and SPME can be used. These chemicals can then be synthesised using synthetic organic chemistry techniques and then field testing can be conducted to determine the best semiochemical(s) to use.

Molecular phylogeny and biogeography of Panorpidae (Mecoptera).

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Panorpidae is the largest family in Mecoptera, covering approximately 70% species of the order. However, the phylogenetic relationship within Panorpidae has not been adequately explored. Here, we analyzed the phylogenetic relationships among 70 species of five genera in Panorpidae using maximum likelihood and Bayesian methods. The results show that the genera *Neopanorpa*, *Sinopanorpa* and *Dicerapanorpa* are monophyletic, while the widespread genus *Panorpa* is paraphyletic. Besides, the origin, dispersal, and divergence of Panorpidae were briefly discussed.

Differential gene expression patterns associated with dispersal ability in the alpine butterfly, *Parnassius smintheus*.

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Dispersal is a critical process affecting the dynamics and persistence of spatially structured

populations. Variation in dispersal phenotype can be caused by changes in DNA sequence or in gene expression. We used RNA−seq technology to prepare an adult transcriptome for the alpine butterfly, *Parnassius smintheus* to (i) identify variation in DNA sequence using candidate locus, Pgi which is involved in providing energy for flight in insects and (ii) compare gene expression patterns among individuals with differing dispersal histories within a network of interconnected populations. We found different gene expression patterns between dispersers and non-dispersers suggesting a degree of functional specialization.

Live yeasts determine development time in Drosophila melanogaster.
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The gut of insects is host to bacteria, archaea, fungi, protozoans and viruses, and together they affect host physiology. Most studies have focused on the bacterial microbiota, but little is known about the other members of the gut flora. The gut microbiota of *Drosophila melanogaster* is relatively simple, and its bacteria and yeast species have been characterized, thus making it a useful system for understanding the role of yeasts in insect physiology. Yeasts are usually regarded as a food source; however, here we show that the effect of yeasts on *D. melanogaster* development is not solely explained by the nutritional content of the yeast.

Prebiotics and probiotics for the control of the intestinal parasitic fungus *Nosema ceranae* in the honey bee (*Apis mellifera*) at the colony level.
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There are many factors contributing to poor honey bee health, one of them being the microsporidian *Nosema ceranae*. *N. ceranae* is a fungal pathogen of the honey bee, damaging the gut cell lining and leading to death. This research will evaluate novel prebiotics and probiotics for their ability to reduce *N. ceranae* infection and improve honey bee health. To validate the efficacy of promising compounds, previously identified in our lab, a field study using full size production colonies is currently being conducted. Preliminary data and conclusions from the first months of the trial will be presented.

Recovery of the endangered mottled duskywing (Hesperiidae: *Erynnis martialis*), a candidate species to re-establish at Pinery Provincial Park.
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The Mottled Duskywing, a rare skipper butterfly, occurs in geographically scattered locations in southern Ontario. Adults associate closely with larval foodplants (*Ceanothus* spp.). The

Mottled Duskywing is "endangered" both federally and provincially, affording it protection under the *Endangered Species Act*. We have collaborated for several years to address key knowledge gaps and recovery actions identified for the species. Our research and survey activities are directed towards increasing existing populations and re-establishing the species where it previously occurred within the province. Currently the best reintroduction site is Pinery Provincial Park where the Mottled Duskywing was last verified in the early 1990s.

Yellow or blue? Analyzing optimal colour of mass trapping of western flower thrips (*Frankliniella occidentalis*) in Canadian greenhouses.

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Mass trapping can be optimized by selecting trap colours pests prefer. For western flower thrips (*Frankliniella occidentalis*), the primary pest of greenhouse crops, literature is varied on whether yellow or blue traps are preferred; blue cards may also reduce impacts on natural enemies. We compared insect trap captures in commercial greenhouses in Southern Ontario in 2017 to determine the optimal mass trap color for greenhouse IPM programs. Results suggest that purported benefits of using blue traps in greenhouses may generally be a fallacy, and that the efficacy of trap color is likely affected by light transmission in different seasons.

Bumblebee risk assessment flawed from tier-one: how disparate methodologies raise questions about comparability of LD50 data.

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Bumblebees differ markedly from honeybees in many aspects of their ecology and life history, and potentially also their sensitivity to pesticides. A first step when examining chemical toxicity for risk assessment is to assay the exposure levels resulting in lethal effects. While such LD_{50} assays are well characterised for honeybees, researchers have variously adapted these methods to suit bumblebees. This methodological variation has produced disparate LD_{50} values across studies using the same active ingredient. Here we summarise these data, and discuss the importance of producing a standardized methodology to obtain accurate and comparable results for risk assessments for *Bombus* species.

Effect of *Varroa destructor* parasitism and sublethal doses of clothianidin on honey bee (*Apis mellifera*) behavior.

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Honey bees (*Apis mellifera*) are exposed to *Varroa destructor* and neonicotinoid insecticides. Previous studies have focused on the effects of these stressors on foraging behaviour and learning, but little is known about their combined effects on behaviours related to social immunity. We assessed the impact of sublethal doses of clothianidin and/or of *V. destructor* on hygienic and grooming behaviour and also on memory retention. The results indicate that sublethal doses of clothianidin and *V. destructor* may affect social immunity and memory retention, although no additive or synergistic effects were found. The implications of these findings will be discussed.

A review of the cleptoparasitic bee genus *Epeolus* Latreille in the Nearctic region.
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Epeolus Latreille (Hymenoptera: Apidae) is a nearly cosmopolitan genus of cleptoparasitic bees that exclusively parasitize polyester bees of the genus *Colletes* Latreille (Hymenoptera: Colletidae). Like many other cleptoparasitic bees, *Epeolus* are difficult to tell apart morphologically and therefore are taxonomically challenging. The genus is most diverse in North America but has never before been revised for the entire continent. To delineate species I used an integrative approach that combines morphology with molecular (CO1) data. Now, at least 40 species are recognized as occurring north of Mexico, 11 of which await formal description.

On the genetic basis of hygienic and overwintering behaviour in the honeybee.
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The recent honeybee decline has been linked to harsh winters and disease but previous attempts to address the issue have fallen short. Both disease reduction and winter survival can be improved by modifying colony behavior. The honeybee can perform hygienic tasks and overwintering behavior, which reduces disease and winter colony loss respectively. Finding the genetic basis of these behaviours would improve our understanding of how these behaviours are governed. We performed a Genome Wide Association Study on honeybee populations across Canada to identify markers that can be used to breed honeybees better equipped to handle disease and harsh winters.

Fantastic pollinators and where to find them.
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Canada is currently behind other countries when it comes to understanding the diversity and distribution of our native wild pollinators. The Raine lab pollinator biodiversity monitoring project aims to address these knowledge gaps, providing baseline and trend data on Ontario

wild pollinator communities. Here, we present preliminary findings for bee data collected, address some of the challenges faced during this project, and plans to expand our focus to other flower-visitor taxa, such as flies, beetles and wasps. These multi-season datasets are crucial to scientists and policy makers when deciding how to conserve pollinators and the essential ecosystem services they provide.

GPA2/GPB5: a novel glycoprotein hormone with an unknown function.
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GPA2/GPB5 and its receptor (LGR1) represent a novel neuroendocrine glycoprotein hormone-signaling system found in both vertebrate and invertebrate genomes, however its function remains elusive. To better delineate the role of GPA2/GPB5 in the mosquito *Aedes aegypti*, we aimed to characterize the expression profile of both GPA2/GPB5 and LGR1, as well as elucidate downstream signaling pathways. All in all, our research supports that, in addition to its putative role in regulating ion and water balance, GPA2/GPB5 may function in reproduction, which in mosquitoes, has direct linkage to the transmission of pathogens like the Zika and dengue viruses.

GWAS for honey production and aggressive behaviour in honey bees.
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For beekeepers the most important traits in honey bees are honey production and aggressive behaviour. We have sequenced 1000 honey bee colonies and measured those phenotypes. With next generation sequencing techniques and modern computational methods allows for the elucidation of associations between the honey bees' genetic markers and these colony level phenotypes, honey production and aggressive behaviour. This represents the first genome wide association study of these traits in honey bees.

Examining changes in the wild bee fauna of south-central Ontario: a long-term analysis of community composition and structure.
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Wild bees provide critical pollination services, supporting the production of food crops and maintaining flowering plant diversity. However, wild bees appear to be declining around the world. Wild bee communities were resampled at four historical study sites in south-central Ontario using a combination of sweep netting and pan trapping methods. Plant-pollinator interactions, and community composition and structure were examined over periods of 15 and 49 years. Further understanding of the historical and current status of wild bees in Ontario provides important insights into how wild bee communities and plant-pollinator

interactions could change as a result of anthropogenic and environmental processes.

Field guide to the hover flies (Syrphidae) of northeastern North America.
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The guide will cover all 414 syrphid species found north of Tennessee and east of the Mississippi. Making a comprehensive guide to a family of insects presents many challenges not present with traditional field guides. Minor to major taxonomic revisions were required for virtually all genera. Complete revisions to two genera were published while other changes will be published in the guide. All but 13 species (3%) will be identifiable without a microscope. We anticipate that this project will open up the syrphid world for ecological work as well as for amateur interests.

"Hearing aids" in Satyrinae butterflies: using laser vibrometry to assess the hearing of Cercyonis pegala.
Sun, P. (C.)*[1], N. Mhatre[2], A.C. Mason[2], and J.E. Yack[1].
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Butterflies belonging to certain subfamilies of Nymphalidae, such as Satyrinae, have tympanal ears located at the base of their forewings. In some species, the ears are associated with unusually large and swollen wing veins ("inflated veins"). We use laser Doppler vibrometry on the Common Wood-Nymph *(Cercyonis pegala*) (Lepidoptera: Nymphalidae) and selected other Wood-Nymphs from Ontario, to test the hypothesis that inflated veins aid in hearing. Our results suggest that inflated veins represent a mechanism of auditory frequency tuning, which may have implications for novel acoustic technology and provide insight into acoustic sensory ecology of butterflies.

Studying the genetic underpinnings of innate immunity behavior in *Apis mellifera* (honey bee).
Tiwari, T.*, C. Kent, H.K. Patel, S. Rosel, K. Dogantzis, A. Dey, and A. Zayed.
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Honey bee is model organism for social behavior and one of the most important managed pollinator in agriculture. The recent losses in their colonies are alarming. They live in crowded nests providing favorable conditions for the spread of infectious diseases. Main causative agents are viruses, bacteria, fungus and parasites. But they have several social and

personal ways for protecting themselves against infections. I plan to carry out genome-wide association studies to get deeper insight in genetics of innate immunity and how pathogen loads differ between colonies. This will set the groundwork for breeding the bees with a stronger innate immune system.

Field realistic neonicotinoid exposure reduces honey bee health.

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Experiments linking neonicotinoids and declining bee health have been criticized for not being 'field realistic'. Here we quantified the duration and magnitude of neonicotinoid exposure in Canada's corn-growing regions and used these data to design realistic experiments. Colonies near corn were naturally exposed to neonicotinoids for up to 4 months—the majority of the honey bee's active season. Realistic experiments showed that neonicotinoids increased worker mortality and were associated with declines in social immunity and increased queenlessness over time. Our work demonstrates that field-realistic exposure to neonicotinoids can reduce honey bee health in corn-growing regions.

Predator diversity and predation rate: results of a mesocosm experiment in the Yukon, Canada.

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Niche complementarity and a sampling effect can lead to a negative relationship between predator diversity and prey abundance, but the relationship is far from simple. We performed experiments in the Yukon, Canada, to investigate the relationship between spider diversity and prey consumption. We carried out feeding trials, from which we found that individual consumption rate increased with spider body size, and that intraguild predation is present. We carried out mesocosm experiments in which we varied the spider diversity of small enclosures. The fewest prey remained in the assemblages with the lowest phylogenetic diversity, which we suggest is due to antagonistic intraguild relationships.

Identification, expression, and functional characterization of the adipokinetic hormone/corazonin-related peptide receptor in *Aedes aegypti.*

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The recently discovered adipokinetic hormone/corazonin-related peptide (ACP) is an insect neuropeptide structurally intermediate between the corazonin (CRZ) and adipokinetic

(AKH) hormones. To date, the function of the ACP signaling system is unclear. In the present study, we identified and characterized the ACP receptor in *Aedes aegypti*. The complete cDNA sequence encoding *A. aegypti* ACPR was isolated, the alternative splicing of which gives rise to three receptor variants. Functional characterization of *Aedae*ACPR-I revealed highly-selective and dose-dependent activation of the receptor by *Aedae*ACP. Analysis of spatial and developmental expression profiles reveals enrichment of *ACPR-I* in the central nervous system and post-eclosion, respectively.

Exploring poisonous mechanism of honeybee, *Apis mellifera ligustica* Spinola, caused by pyrethroids.

Wang, Q.[1,2], E. Guzman-Novoa[3], T. Zhou[1,2] , P. Dai[1,2] , Y. Wu[1,2] and Q.-Y. Diao*[1,2].
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This study investigated the effect of Deltamethrin(DM) on calcium channel in the brain nerve cells of adult workers of *Apis mellifera ligustica* Spinola that were cultured in vitro. The results showed that the intracellular calcium concentration was significantly elevated with the DM. Further testing revealed that the target of DM on toxicity of honeybee is the T-type voltage-gated calcium channels. Combining the results of past studies that DM may cause swarming, feeding, learning and acquisition and other behavioral abnormalities, suggesting that the DM may act on T-type calcium channel in brain cells of honeybees and result in abnormalities.

Ontogeny of social living in caterpillars and the role of vibratory communication.

Yadav, C.* and J.E. Yack.
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Sociality is widespread among larval insects, with many species switching from social early instars to solitary late instars. However, there is not much known about "how" this occurs. We identified larval stages based on morphological features and our study shows that masked birch caterpillar changes from social living in first two instars, grouping together and building communal shelters, to solitary in last two, and third instar is transitional. We also tested hypotheses on role of vibrations in social recruitment in early instars and territoriality in late instars, providing first ever evidence of vibroacoustics mediating sociality in caterpillars.

Flower Fly (Diptera: Syrphidae) phylogenetics and beyond: challenges and opportunities presented by next-gen sequencing.

Young, A.D.*[1,2], K. Moran[1,2], J. H. Skevington[1,2], T. R. Burt[3], X. Mengual[3], G. Ståhls[4], K. Jordaens[5], S. Bot[6], M. Reemer[7], M. Hauser[8], M. van Zuijen[9], C. Palmer[10], J. van Steenis[7], W. van Steenis[7], A. Ssymank[11], G. F. G. Miranda[12], F. C. Thompson[13], K. M. Bayless[14], B. Wiegmann[14], and B. Cassel[14].

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With "next-gen" sequencing methods becoming more accessible and affordable by the day, the field of phylogenetics is undergoing a data revolution. For the first time in the history of the discipline, data management has become arguably a larger challenge than data collection itself. Using our recently published syrphid phylogeny as a basis, we have expanded our taxon and gene sampling many-fold for several ongoing projects. These include Sanger sequencing of novel genes based on NGS-led data exploration, a more thoroughly sampled hybrid enrichment project lead by Trevor Burt and Ximo Mengual, and a phylogenetic analysis of the lower Cyclorrhapha.

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CONTENTS

I. ARTICLES

M. A. SMITH, J. V. MCDONALD, M. KENT, E. MORRIS, and J. C. TRAGER — The use of social media to document a range extension of an iconic social parasite ant.....1–5

F. DALLAIRE and M. CUSSON — Comparative toxicity of the nonsteroidal ecdysone agonists tebufenozide and methoxyfenozide to early and late larval instars of the white-marked tussock moth, *Orgyia leucostigma*.....6–12

O. LONSDALE — *Phytomyza krygeri* Hering (Diptera: Agromyzidae), a new invasive species for North America on columbine.....13–21

C. ADLAM, E. DESPLAND, and F. BEAULIEU — Spatial and seasonal patterns of ground beetle (Coleoptera: Carabidae) activity across habitats at the Mont St. Hilaire Biosphere Reserve, Quebec.....23–38

T. J. PRENEY and R. J. L. JONES — A New Family to Canada with the Discovery of the Pleasing Lacewing *Nallachius americanus* (McLachlan) (Neuroptera: Dilaridae) at the Ojibway Prairie Complex in Windsor, Ontario.....39–41

II. POSTER AND PRESENTATION ABSTRACTS — ENTOMOLOGICAL SOCIETY OF ONTARIO ANNUAL GENERAL MEETING.....43–69

III. ESO OFFICERS AND GOVERNORS 2017–2018.....70

IV. ESO OFFICERS AND GOVERNORS 2016–2017.....inside front cover

V. FELLOWS OF THE ESO.....inside back cover

VI. APPLICATION FOR MEMBERSHIP.....inside back cover

VII. NOTICE TO CONTRIBUTORS.....inside back cover

JOURNAL OF THE ENTOMOLOGICAL SOCIETY OF ONTARIO • VOLUME 148, 2017